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IDENTIFICATION OF TESTOSTERONE IN HUMAN SPERMATIC VEIN BLOOD*

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THE assumption that testosterone is secreted by the human testis is based on good but indirect evidence. Testosterone is the most potent naturally-occurring androgen and has been isolated from extracts of the testis (1). Isolation of a hormone from a glandular extract, however, does not necessarily imply secretion from the gland. Additional evidence is provided by the fact that the administration of testosterone will prevent the involution of the accessory sex organs and secondary sex characteristics which otherwise occurs in men and in male animals following castration. Such biologic activity, however, is shared by several steroids, some of which have also been isolated from extracts of testes.

With the development of sensitive chemical methods for the identification of steroids in blood, an attempt was made to characterize the secretory products of the human testis by analyzing blood from the spermatic veins and arteries of patients with prostatic cancer undergoing surgical castration. Differences in steroid content between the arterial and venous testicular blood were employed to determine which hormones were secreted by the testis. This report is concerned with the identification of tes-

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tosterone in human spermatic vein blood. The fact that testosterone was found in the venous blood and was not present in the arterial blood provides strong evidence that this steroid is a true secretory product of the human testis.

MATERIALS AND METHODS

Clinical material. These studies were carried out on 8 previously untreated patients with carcinoma of the prostate. The ages of the patients ranged from 50 to 74 years, with an average of 62.6 years.

Clinical methods. Bilateral orchiectomy was performed as a therapeutic procedure on all 8 patients. Prior to removal of the testes, both spermatic cords were exposed through low bilateral inguinal incisions and the spermatic arteries and veins dissected free. A suitable vein was severed on each side and venous blood from the testes collected in heparin. An approximately equal volume of blood from the spermatic artery of each patient was collected and analyzed in an identical manner and in parallel with the venous blood. Arterial-venous differences were used to establish which hormones were secreted by the testis. From the 8 patients a pooled total of 1485 cc. of blood was collected from the spermatic veins and 1752 cc. from the spermatic arteries.

Analytical methods. Immediately upon collection in the operating room, each blood sample was poured into a centrifuge bottle containing 4.5 volumes of 95 per cent ethanol. The mixture was stirred vigorously for ten minutes, after which the precipitated blood proteins were removed by centrifugation. The protein precipitates were washed three times with hot absolute ethanol and discarded. The original alcoholic supernatant liquid and the alcohol washes were combined and the alcohol removed by vacuum distillation at 35° C. The aqueous residue was extracted five times with 500 cc. of ether to obtain the free, unconjugated steroids. The remaining aqueous phase was hydrolyzed by refluxing at pH 1 for thirty minutes, and extracted with ether. This fraction, containing the conjugated steroids, was saved for future analysis.

The unconjugated steroids were dissolved in toluene and the phenolic fraction extracted with 1N NaOH. The neutral fraction remaining in the toluene was obtained in the conventional manner, "defatted" by partitioning between hexane and 70 per cent EtOH, and separated into neutral ketonic and neutral non-ketonic fractions by means of Girard's reagent. At this stage, all similar neutral fractions from individual patients were combined into respective pools for arterial and venous blood.

RESULTS

The neutral ketonic fractions from arterial and venous blood were analyzed separately in parallel for testosterone by means of paper chromatography, using methods developed by Zaffaroni (2) and Bush (3), and by countercurrent distribution as described by Engel, Slaunwhite, Carter and Nathanson (4).

A preparative paper chromatogram was used first to achieve further purification. The neutral ketonic fraction of venous blood was applied to a 10-cm. starting line and the paper chromatogram developed for sixty hours with cyclohexane saturated with propylene glycol as the mobile phase, and propylene glycol as the stationary phase. Testosterone reference standards

were run simultaneously in the same tank. After sixty hours, the testosterone standards had moved 7.5 cm. from the starting line, as detected by scanning the dried paper chromatogram in ultraviolet light. There was ultraviolet-absorbing material on the unknown paper chromatogram which had moved the same distance from the starting line as the testosterone reference standards. This area of paper was cut out, eluted with methanol and re-applied to a second paper chromatogram, which was developed for twenty hours with cyclohexane and toluene (1:1 by volume) using propylene glycol as the stationary phase. The dried paper chromatogram was scanned in ultraviolet light and an area that absorbed ultraviolet light was observed 29 cm. from the starting line, which corresponded with the distance moved by the testosterone reference standards (Fig. 1).

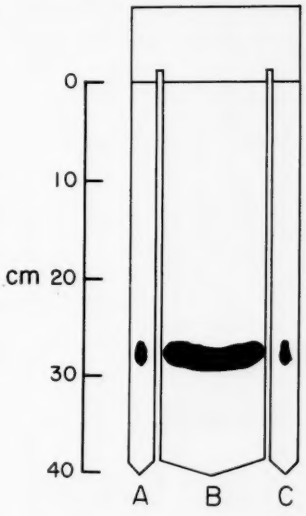

FIG. 1. Diagram of the paper chromatographic analysis for testosterone in spermatic vein blood by the method of Zaffaroni. **A** and **C** = testosterone reference standards; **B** = extract from spermatic vein blood. The paper chromatogram was developed for twenty hours with cyclohexane and toluene (1:1 by volume), using propylene glycol as the stationary phase.

The testosterone zone was eluted from the second paper chromatogram and analyzed by countercurrent distribution in a 100-tube, all-glass countercurrent distribution instrument designed by Craig, Hausmann, Ahrens and Harfenist (5). The composition of the solvent system was 60 per cent ethanol; 40 per cent water: 50 per cent ethyl acetate; 50 per cent cyclohexane. The equivalent amount of testosterone in each tube after a 100-transfer distribution was measured by the absorption in the ultraviolet range at 240 mμ. The resultant countercurrent distribution curve is shown in Figure 2. The analysis curve agreed well with the theoretical curve for testosterone calculated by the method of Craig and Craig (6), yielding additional evidence for the existence of testosterone in testicular venous blood.

Blood from the spermatic artery was analyzed in an identical manner and in parallel with blood from the spermatic vein in order to detect arterial-venous differences in steroid content. Paper chromatography by Zaffaroni's technique failed to reveal any ultraviolet-absorbing material similar to testosterone in the arterial blood. The fractions from the paper chromatograms of the arterial blood which should have contained any testosterone present were analyzed by countercurrent distribution in the same way as those of venous blood. The countercurrent distribution curve obtained is plotted in Figure 2, for contrast with that obtained from the venous blood. Comparison of the two curves reveals the probable ex-

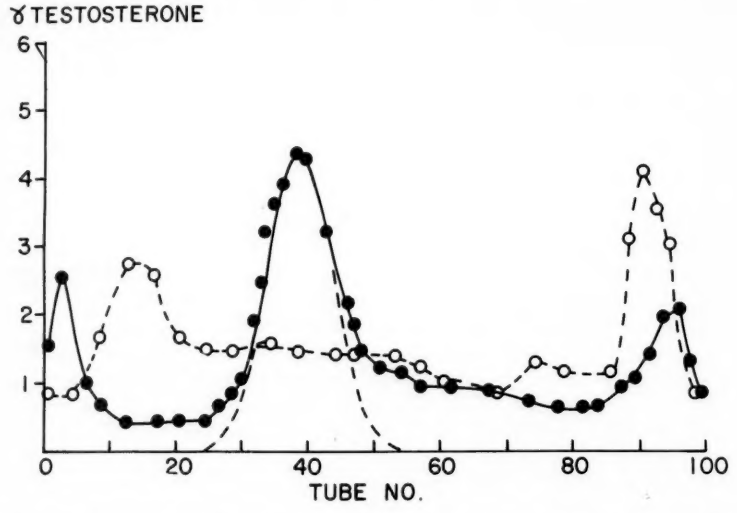

FIG. 2. Countercurrent distribution analysis for testosterone in spermatic arterial and venous blood. Broken line with circles = arterial blood; solid line with dots = venous blood; plain broken line = theoretical curve for testosterone. Solvent system: 60 per cent ethanol; 40 per cent water: 50 per cent ethylacetate; 50 per cent cyclohexane. Partition coefficient (K) for testosterone, 0.7.

istence of testosterone in venous blood from the testes, but not in the arterial blood flowing to the testes. This arterial-venous difference is good evidence that the testes secreted testosterone into the bloodstream.

The peak tubes from the countercurrent distribution of the venous blood sample were combined and analyzed further by a method of paper chromatography developed by Bush (3), which involves different principles and solvents from those used in the Zaffaroni method. A biphasic solvent system composed of 100 per cent petroleum ether: 80 per cent methanol and

20 per cent water was used. After an equilibration period of twenty-four hours at 23 ± 1° C., the paper chromatogram was developed with the upper phase of this solvent system for twenty-four hours. Testosterone reference standards were run simultaneously.

An ultraviolet photograph of the resulting paper chromatogram is shown in Figure 3. As can be seen from the photograph, the middle spot—the sample from spermatic vein blood—had moved the same distance from the starting line as the testosterone reference standards on each side, thus providing additional evidence of the presence of testosterone.

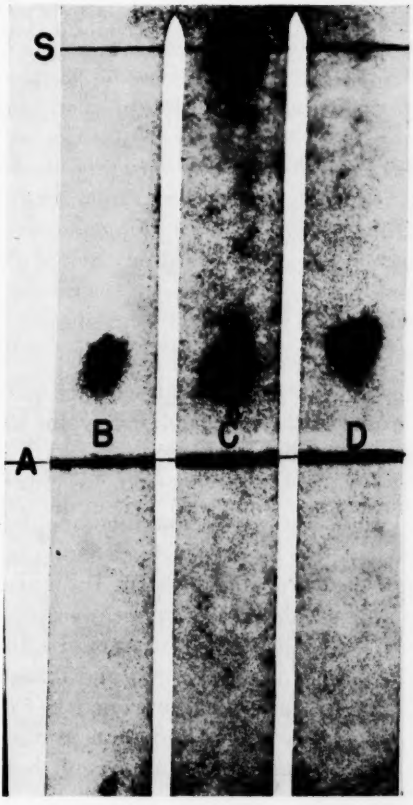

FIG. 3. Ultraviolet photograph of the paper chromatographic analysis of spermatic vein blood for testosterone by the method of Bush. **S** = starting line; **A** = artifact line; **B** & **D** = 5γ testosterone reference standard; **C** = spermatic vein extract.

It was estimated that the amount of testosterone in this fraction was approximately 10 micrograms, judging by the degree of absorption of ultraviolet light in comparison with the testosterone standards.

The material corresponding to testosterone was eluted from the paper chromatogram, dissolved in abolute ethanol, and analyzed for androgenic activity by topical application to the combs of baby chicks, using the biologic assay method of Rakoff, Paschkis and Cantarow (7) as modified by Munson and Sheps (8). The results in comparison with controls and standards are shown in Table 1. The difference between the effect of the eluate and of ethanol alone was significant at $P < 0.001$ by the *t*-test. The potency of the total eluate, estimated statistically in the usual manner, was equivalent to 6.0 micrograms of androsterone, with 68 per cent confidence limits of 3.5 to 11.0 micrograms, and 95 per cent confidence limits of 2.0 to 19.0 micrograms. In this biologic assay method, testosterone is 39 per cent as active as androsterone

TABLE 1. RESULTS OF BIOASSAY OF FINAL PRODUCT FROM HUMAN SPERMATIC VEIN BLOOD FOR ANDROGENIC ACTIVITY

Test material	No. of chicks	$100 \times \log \frac{\text{Mean response comb. wt., mg.}}{\text{body wt., dkg.}}$
Eluate	4	78.5
Ethanol only	10	54.3
Androsterone, 0.1 μg.	24	76.2
Androsterone, 0.8 μg.	23	99.6

Standard deviation for the assay, 11.2. Lambda for androsterone, 0.43.

(68 per cent confidence limits: 32 to 48 per cent). Therefore, the total androgenic activity of the eluate was approximately equivalent to 15 micrograms of testosterone. This value agreed well with the amount estimated by ultraviolet absorption.

DISCUSSION

In this investigation, a compound has been obtained from human spermatic vein blood which is soluble in organic solvents, neutral and ketonic by the Girard T reaction. Its movement in three sequential analyses by paper chromatography in three different solvent systems was identical with that of authentic testosterone. Its distribution curve by countercurrent distribution, using a fourth and different solvent system and 100 transfers, agreed with the theoretical curve for testosterone. The final product was a biologically active androgen by the chick comb test. Furthermore, there was reasonably good agreement between the amount of testosterone estimated to be present by the biologic assay method and that determined by ultraviolet absorption. On the basis of this evidence, it was concluded that this androgenic compound in human spermatic vein blood is testosterone and, because none was found in arterial blood flowing to the testes, that testosterone is secreted into the blood stream by the human testis *in situ*.

It is not surprising that testosterone should be found in human spermatic vein blood, since it has been identified previously in the testicular venous blood of dogs by West, Hollander, Kritchevsky and Dobriner (9), and in perfusates of human testes by Savard, Dorfman and Poutasse (10). Both groups of investigators demonstrated that Δ^4-androstenedione-3,17 was also a secretory product of the testis under the conditions of their respective studies. It has been postulated on the basis of physiologic observations that the testis may secrete other hormones besides testosterone which may regulate the secretion of gonadotropins by the anterior pituitary (11). For this reason, analyses of the phenolic and other neutral fractions are planned.

This type of investigation may also contribute to a better understanding of the role of the testis in cancer of the prostate. Since androgen is essential to the normal growth and activity of the prostate and to the optimal growth of at least some neoplastic prostatic tissue, the beneficial effects of castration in patients with cancer of the prostate are usually attributed to the removal of the principal source of androgen from the body. Five of the patients in this study obtained clear-cut objective remissions following castration; 3 had possible objective remissions; and all 8 had definite subjective improvement. The demonstration that the testes in these old men with prostatic cancer produced testosterone lends support to the thesis that androgen withdrawal accounts for the beneficial effects of castration, but does not prove it. Observations by Brendler, Chase and Scott (12) and Hollander, West, Whitmore and Pearson (13), concerning the administration of testosterone to patients with metastatic prostatic carcinoma, indicate that testosterone may fail to exacerbate the disease in some cases and may produce subjective remissions in others. Such observations suggest that the mechanism of castration remission may involve more than simple androgen withdrawal, at least in some patients. It is conceivable that the testis may secrete hormones besides androgens which are also involved in the genesis and/or growth of prostatic cancer. Isolation studies on testicular venous blood should help to provide an answer to this question.

SUMMARY

Testosterone was identified in spermatic vein blood from patients with carcinoma of the prostate by means of multiple sequential analyses by paper chromatography and countercurrent distribution and by bioassay methods. Parallel analyses carried out on blood from the spermatic arteries of the same patients revealed no testosterone. The conclusion is drawn from these observations that testosterone is a true secretory product of the human testis.

Acknowledgments

The final product was tested for biologic activity in the Endocrine Research Laboratory, Harvard School of Dental Medicine, through the courtesy of Dr. Paul L. Munson. The authors acknowledge with gratitude the technical assistance of Miss Barbara Damast and Mrs. Louise Berman. Gratitude is expressed to Dr. T. F. Gallagher for his advice and assistance in this study.

REFERENCES

1. David, K. E.; Dingemanse, E.; Freud, J., and Laqueur, E.: Über krystallinisches männliches Hormon aus Hoden (Testosteron) wirksamer als aus Harn oder aus Cholesterin bereitetes Androsteron, *Ztschr. f. physiol. Chem.* **233**: 281, 1935.
2. Zaffaroni, A.: Micromethods for the analysis of adrenocortical steroids, *Recent Progr. Hormone Res.* **8**: 51, 1953.

3. BUSH, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues, *Biochem. J.* **50:** 370, 1952.
4. ENGEL, L. L.; SLAUNWHITE, W. R., JR.; CARTER, P., and NATHANSON, I. T.: The separation of natural estrogens by countercurrent distribution, *J. Biol. Chem.* **185:** 255, 1950.
5. CRAIG, L. C.; HAUSMANN, W.; AHRENS, H., JR., and HARFENIST, E. V.: Automatic countercurrent distribution equipment, *Analyt. Chem.* **23:** 1236, 1951.
6. CRAIG, L. C., and CRAIG, D.: Extraction and distribution, *in* Weissberger, A.: A Technique of Organic Chemistry. New York, Interscience Publishers, 1950, vol. 3, p. 171.
7. RAKOFF, A. E.; PASCHKIS, K. E., and CANTAROW, A.: Androgen assay on 3-day-old chicks, *Proc. Soc. Exper. Biol. & Med.* **55:** 124, 1944.
8. MUNSON, P. L., and SHEPS, M. C.: The biological assay of androgens, *in* Conference on Comparison of the Biological Properties of Steroids and Hormones, ed. by Wermer, P. L. Chicago, American Medical Association (in press).
9. WEST, C. D.; HOLLANDER, V. P.; KRITCHEVSKY, T. H., and DOBRINER, K.: The isolation and identification of testosterone, Δ^4-androstenedione-3,17, and 7-ketocholesterol from spermatic vein blood, (abstract) *J. Clin. Endocrinol. & Metab.* **12:** 915, 1952.
10. SAVARD, K.; DORFMAN, R. I., and POUTASSE, E.: Biogenesis of androgens in the human testis, (abstract) *J. Clin. Endocrinol. & Metab.* **12:** 935, 1952.
11. MCCULLAGH, E. P.: Sex hormone deficiency—Some clinical considerations, *Recent Progr. Hormone Res.* **2:** 295, 1948.
12. BRENDLER, H.; CHASE, W. E., and SCOTT, W. W.: Prostatic cancer: further investigation of hormonal relationships, *Arch. Surg.* **61:** 433, 1950.
13. HOLLANDER, V. P.; West, C. D.: WHITMORE, W. F., JR., and PEARSON. O.: Unpublished data.

ALDOSTERONE EXCRETION IN PREGNANCY*

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FOLLOWING the isolation and characterization of the salt-retaining factor of the adrenal cortex by Simpson *et al.* (1), considerable interest has been aroused regarding its regulatory action on electrolyte and fluid balance. The original observation of Deming and Luetscher (2) that this hormone, now called aldosterone, is excreted in increased amounts in edematous conditions has been confirmed by other investigators. In earlier studies, Chart *et al.* (3), Venning *et al.* (4) and Gordon *et al.* (5) found that patients with toxemia of pregnancy excreted relatively large amounts of a sodium-retaining factor, whereas in the normal gravid woman amounts only slightly above those in the non-pregnant state were observed. Recently, using acid and β-glucuronidase hydrolysed urine, Venning and Dyrenfurth (6) reported that normal pregnant women at term excrete from 16 to 34 micrograms (μg.) of aldosterone per day in comparison with the normal range of from 1 to 6 μg. per day for non-gravid women. In the present study we have measured aldosterone excretion throughout gestation and have attempted to correlate the output of this hormone with clinical findings.

METHODS AND CLINICAL MATERIAL

Aldosterone. The bioassay of aldosterone was carried out by a modification of the Singer-Venning method (7) which measures the effect of this hormone upon sodium excretion in the adrenalectomized rat. A 10-hour aliquot of urine acidified to pH 1.5 was extracted immediately with chloroform and again after standing at room temperature for twenty-four hours. The extracts were washed with small amounts of weak alkali and water. The residue was applied to a paper strip and was chromatographed in the Bush B5 system at room temperature. The aldosterone fraction was eluted and submitted to bioassay. Standard amounts of desoxycorticosterone acetate were run with the test. In Patient S.B. (Case 4) the urinary extracts were assayed at 10-minute and 30-minute volume levels; the other cases were assayed at the 30-minute volume dose only.

17-Hydroxycorticosteroids. The urinary corticoids were determined by the Silber-

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Porter method following β-glucuronidase hydrolysis (8). The standard used was hydrocortisone.

Case 1

A.F. This 32-year-old woman, para i, gravida ii, had a normal course throughout pregnancy (Fig. 1). The total gain in weight was 34 pounds, and in the last trimester she was restricted with regard to table salt. During this period her sodium intake was approximately 100 mEq. per day. The delivery was normal except for a retained placenta, which was removed manually.

On the forty-second day of pregnancy, the aldosterone excretion was within the normal range, it was slightly higher on the seventy-second day and on the one hundred and

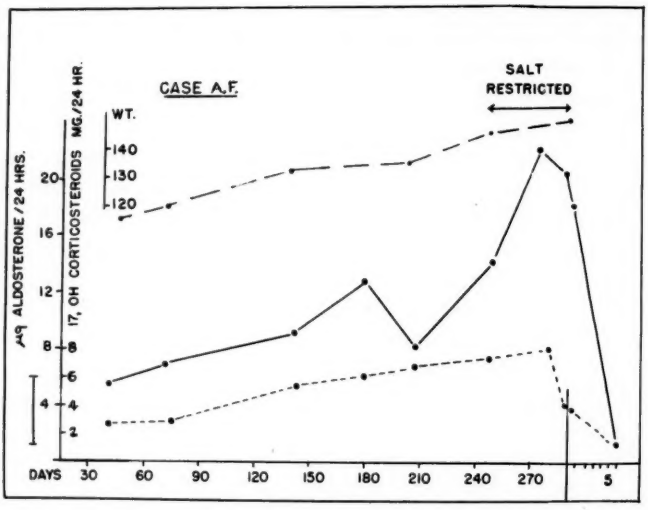

FIG. 1. *Case 1.* Excretion of aldosterone and 17-hydroxycorticosteroids in a normal pregnant woman. Parturition occurred on day 289. The upper curve represents weight, the middle curve aldosterone excretion, and the lower curve 17-hydroxycorticosteroid excretion.

forty-fifth day it was definitely above normal range. From then on it gradually increased until twelve days before delivery it had reached a peak of 22 μg. per day. It remained at a high level until the day after delivery. Five days later it had decreased to a level of 4 μg. per day. The moderate salt restriction carried out in the last trimester may have caused the further increase in aldosterone excretion. However, in other cases of normal pregnancy studied for short periods of time near term, similarly high values have been observed. There was no clinical edema nor increase in blood pressure throughout gestation in this patient. The laboratory findings were within normal range. The urinary 17-hydroxycorticosteroids increased progressively throughout pregnancy, reaching a level of 8.0 mg. daily six days before parturition. Following delivery the excretion decreased to 1.4 mg. daily.

Case 2

R.S. This patient, aged 35, para i, gravida iv (Fig. 2), was given progesterone intramuscularly for thirteen weeks (beginning on the forty-sixth day of pregnancy), because of low pregnanediol excretion and a history of early miscarriages. Following this treatment the pregnancy appeared to have a normal course. The patient had a depressive psychosis, which improved as the gestation progressed. There was no edema and no increase in blood pressure. Up to the two hundred and fiftieth day, the total gain in weight was $26\frac{1}{4}$

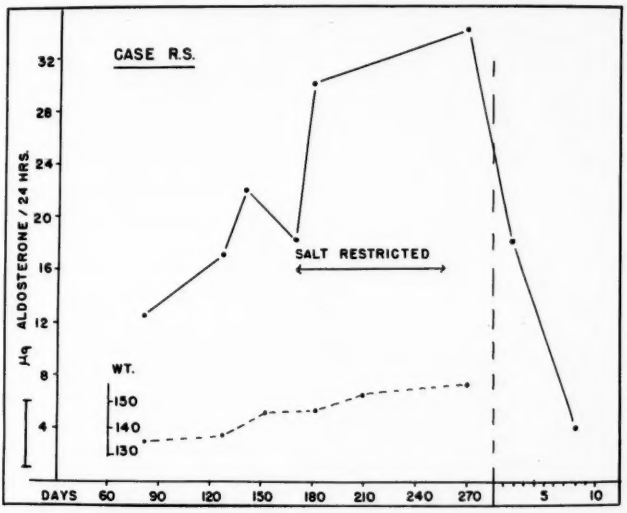

FIG. 2. *Case 2.* Excretion of aldosterone in pregnancy. Parturition occurred on day 280. The upper curve represents aldosterone excretion, and the lower curve, body weight.

pounds. Following this period there was a loss of 2 pounds. Parturition occurred on the two hundred and eightieth day.

Because the patient had shown a tendency to retain fluid in her previous pregnancy, moderate salt restriction was carried out from the one hundred and eightieth day onwards.

On the eighty-seventh day aldosterone excretion was already well above the normal range, being 12 μg. per day. It increased progressively throughout gestation, reaching a maximum of 34 μg. per day, two weeks before delivery. Two days post partum it had decreased to 18 μg. per day and six days later was down to 4 μg. daily.

Case 3

E.A. was a primipara aged 21 (Fig. 3). In the early phase of this preganacy, spotting occurred on and off for the first two months. The pregnanediol levels were within normal limits. From the one hundred and fortieth to the one hundred and eightieth day there was a rapid gain in weight, and at the end of this period some edema was present. From the two hundred and fortieth day onward the patient was given a diet containing 1800

calories, with no added salt allowed. In spite of this, excessive weight gain continued. As this patient was unreliable, it is probable that the dietary instructions were not followed. From the one hundred and nineteenth to the one hundred and sixty-second day the aldosterone output ranged from 16 to 21 μg. per day—values well above the normal range. With the appearance of edema, the daily aldosterone excretion rose to 29 μg. Following this, and coincident with sodium restriction, the aldosterone output gradually decreased, reaching a level of 10 μg. daily nine days before delivery. During this period there was increasing hydramnios and evidence of water retention. In this case the aldosterone fraction was assayed only at one level—a 30-minute volume dose. In other patients studied we have evidence to indicate that there is a substance running with aldosterone on the paper chromatogram which causes sodium excretion, and it is possible that the presence of this substance might have interfered with the bioassay of aldosterone in this patient at this dose level. It has been found that the unidentified material can be diluted out at the 10-minute volume dose.

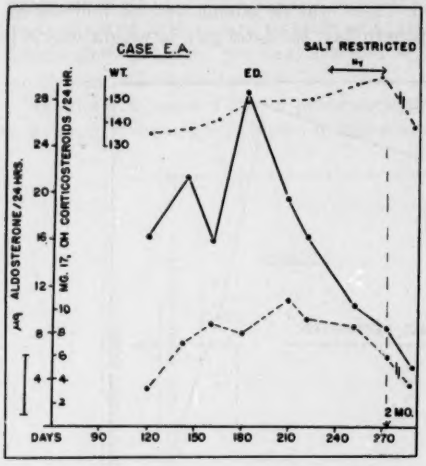

FIG. 3. *Case 3.* Excretion of aldosterone in pregnancy. Parturition occurred on day 271. The upper curve represents weight, the middle curve aldosterone excretion, and the lower curve 17-hydroxycorticosteroid excretion. ED. = edema.

Following delivery aldosterone excretion decreased further, and at two months post partum was down to 5 μg. per twenty-four hours. The urinary 17-hydroxycorticosteroids increased throughout pregnancy, and in the last trimester fluctuated between 8.6 and 10.8 mg. per twenty-four hours. Following delivery, there was the usual decline observed in other cases.

Case 4

S.B. was a woman aged 35, para i, gravida ii (Fig. 4). In the first pregnancy this patient had shown an abnormal retention of fluids and was thought to be toxic. A cesarian section was performed because of inability to control the edematous state near term.

In the pregnancy concerned in this report, the patient was gaining excessive amounts of weight at the time the study was begun. Diamox was given for one week and she lost 7 pounds, but regained this weight within one week after stopping the medication.

When the study was begun on the one hundred and eighty-ninth day of pregnancy, the patient was receiving a low caloric diet, and salt intake was approximately 83 mEq. per day. No edema was present, and values for aldosterone output were 13.1 μg. and 7.2 μg. per day. The low caloric diet was then discontinued, although salt was still restricted. Increased fluid retention and slight edema occurred and hydramnios became apparent. The aldosterone excretion was 15.8 μg per day at this time. Following this the swelling of the hands and face increased and gross hydramnios was evident by the two

hundred and fifty-second day. Aldosterone excretion increased to 31 μg. per day and her condition remained unchanged.

The date of conception was known in this patient. Judging from x-ray studies, the fetal skeletal development of the baby was about four weeks delayed. Because of this, cesarian section was not carried out until the two hundred and eighty-third day. During the first forty-eight hours after birth the baby excreted 1.0 μg. of aldosterone daily, and two weeks later a similar amount was present in the urine.

During the last part of the pregnancy there was considerable fluctuation in the output of aldosterone. On the day of operation it was 28 μg per day; it decreased to 24 μg. on

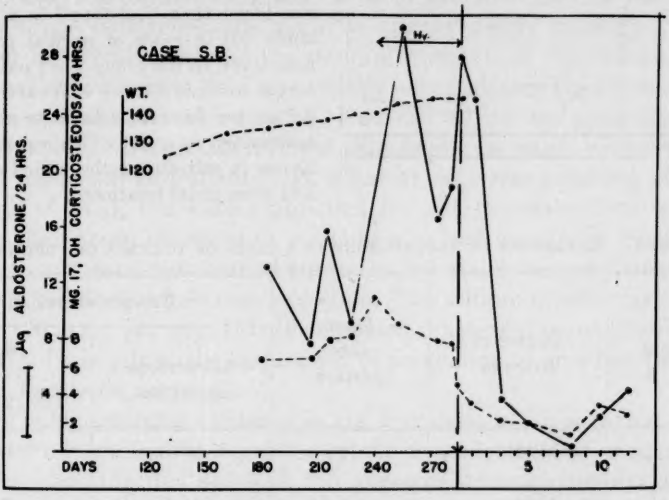

FIG. 4. *Case 4.* Excretion of aldosterone in a pregnant woman who was retaining excessive amounts of fluid. The upper curve represents weight, the middle curve aldosterone excretion, and the lower curve 17-hydroxycorticosteroid excretion. Cesarian section was carried out on day 283.

the first postoperative day; and on the third postoperative day it was down to 3.8 μg. It remained around this level for the remainder of the study.

The urinary 17-hydroxycorticosteroids varied between 6.6 and 11.6 mg. per day during the last trimester and between 1.4 and 3.4 mg. per day in the postpartum period.

Case 5

J.S. This 30-year-old pregnant woman, para ii, gravida iii (Fig. 5), had a normal salt intake, and one week before delivery was excreting 34 μg. of aldosterone per day. The daily aldosterone was measured during the postpartum period. On the second postpartum day, it had decreased to 5 μg. per day, and fluctuated between this level and 1 μg. during the period of study.

Toxemia of pregnancy (8 patients)

Aldosterone and 17-hydroxycorticosteroid excretions were measured in 8 patients

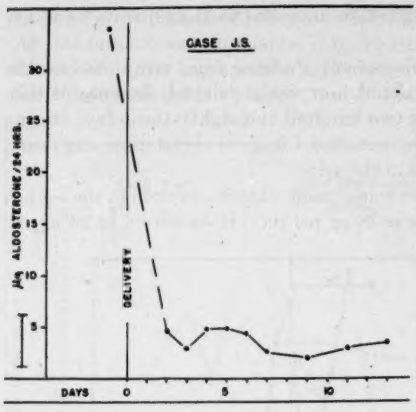

FIG. 5. *Case 5.* Aldosterone excretion ante partum and post partum, in a normal pregnant woman.

with toxemia of pregnancy of varying degrees of severity. The results are shown in Table 1.

Although the aldosterone output was increased above that in the nonpregnant state, the average daily output of 14.7 μg. was lower than that found in a previous study (6) in cases of normal pregnancy near term. In the group was 1 patient with severe toxemia (M.S.) who excreted only 3.7 μg. per day. All 8 patients were being maintained on a diet which was moderately low in salt during the period of study, and were under treatment.

TABLE 1. EXCRETION OF ALDOSTERONE IN 8 CASES OF TOXEMIA OF PREGNANCY

Patient	Degree of toxemia	Days ante partum	Urinary excretion	
			Aldosterone (μg./24 hrs.)	Corticoids, S−P (μg./24 hrs.)
G.S.	Mild	1	18.0	8.8
P.S.	Mild	2	11.0	7.6
K.M.	Moderate	8	7.4	4.6
		6	13.0	10.0
		3	31.0	4.0
		1	31.2	12.8
C.G.	Moderate	19	19.2	18.4
J.McK.	Moderate	10	10.1	3.8
		9	8.8	2.6
		8	5.7	3.6
		7	5.2	1.2
G.J.	Severe	1	14.5	3.0
M.S.	Severe	1	3.7	6.4
G.Z.	Severe	14	24.4	7.6
		12	18.2	8.8

DISCUSSION

The evidence obtained from these studies clearly demonstrates that there is a rise in the excretion of aldosterone in pregnancy and that following parturition these values decrease rapidly and reach normal levels in the early postpartum period.

In investigations not reported here, in which daily measurements of aldosterone excretion were made in the two-week period prior to delivery, considerable fluctuation occurred, so it is possible that in the present studies single determinations made at approximately monthly intervals may not have truly reflected the trend of excretion of this hormone. In 2 of the patients in whom fluid was rapidly accumulating there was an associated rise in aldosterone excretion. However, in the few cases studied, it was not possible to correlate levels of aldosterone excretion with the degree of fluid retention. In Patient S. B. (Case 4), who was retaining abnormal amounts of fluid, the values obtained for aldosterone output were not greater than those observed in patients who had no clinical edema. The moderate salt restriction carried out in Cases 1 and 2 was not thought to be sufficient to affect aldosterone excretion. The sodium intake was approximately 100 mEq. per day. It is the common practice of many obstetricians to restrict table salt in the last month of pregnancy, even when the gestation is considered normal.

There is considerable evidence in the literature to suggest that sodium and water are retained to a greater extent in the latter part of pregnancy, and that when toxemia develops this abnormality is further exaggerated. There is a rise in total body water (9), plasma volume (10, 11) and extracellular fluid (12), and associated with these changes there is a retention of sodium (13). Gray and Plentl (14) showed an absolute gain in sodium space from the beginning of pregnancy until term.

Other hormones besides aldosterone are increased in the latter part of pregnancy. It has been well established by various methods that there is a rise in the level of excretion of adrenocortical steroids (15, 16) and studies on blood have confirmed these findings (17, 18). Progesterone and estrogens are also greatly increased, and all these various hormones may contribute in part to a retention of sodium and a consequent expansion of body fluid (19).

It is not possible with our present knowledge regarding factors that control the release of aldosterone from the adrenal cortex to determine whether the increase of aldosterone in pregnancy is the cause or the result of fluid and sodium retention. In the healthy non-gravid subject a depletion of body sodium, caused either by lowered intake (20) or by loss of body sodium (21), results in a rise in aldosterone excretion. Changes in body

fluid volume affect aldosterone secretion independently of sodium. An expansion of body fluid volume caused by the administration of vasopressin (Pitressin) will in turn lower the output of aldosterone (22, 23), whereas a reduction in the body fluid volume brought about by dehydration will result in a rise in aldosterone excretion (24, 25). In pregnancy, however, a rising aldosterone excretion is observed associated with sodium retention and an expanding body fluid space, which may further aggravate this condition. The stimulus which gives rise to the increased aldosterone output is not known at the present time.

A similar situation is observed in patients with nephrosis, cardiac failure or the ascites of liver disease, associated with edema. Mach *et al.* (26) in a recent paper have suggested that in these conditions the rise in aldosterone is "secondary to the diseased state" and that the factor accounting for this increased secretion may be a "decrease in effective circulating fluid frequently found in these states in spite of the presence of edema." Whether such a mechanism operates in pregnancy is unknown.

The role of the placenta in the elaboration of adrenocortical steroids has not yet been clarified. Although adrenal steroids (27, 28) as well as aldosterone (29) and corticotropin (30, 31) have been isolated from placenta, there is still no conclusive proof that this organ is capable of secreting these hormones.

The results obtained on the patients with toxemia are in disagreement with results of our earlier studies, in which only the free aldosterone was measured. The extracts at that time were not purified by paper chromatography and the total neutral fraction was assayed for its sodium-retaining activity. In those studies the patients with toxemia of pregnancy excreted greater amounts of this factor than did the normal pregnant women. Using acid for hydrolyzing the conjugate of aldosterone, it is now seen that the nontoxic pregnant woman excretes as much as, or even more aldosterone than the toxic patient in spite of the fact that the latter is being maintained on a lower salt intake.

The secretion of increased amounts of aldosterone, a hormone which has a potent sodium-retaining effect, must aggravate a condition in which sodium and fluids are already being retained in the body. Before any conclusions can be drawn, however, regarding the importance of aldosterone in the development of this syndrome, toxic patients will have to be studied over longer periods of time and before any dietary restrictions have been imposed.

SUMMARY

1. Aldosterone excretion has been measured at approximately monthly intervals in 4 pregnant women, and near term in 8 patients with toxemia

of pregnancy. The aldosterone fraction was obtained after prolonged acid hydrolysis at room temperature. The extracts were purified by paper chromatography prior to assay.

2. Pregnancy was associated with an increase in excretion of aldosterone, and following delivery there was a rapid decrease in the output of this hormone.

3. The active accumulation of fluid in 2 of the patients was associated with a rising level of aldosterone output. The levels reached, however, were not higher than those observed in pregnant women without clinical edema.

4. In the patients with toxemia of pregnancy, the amount of aldosterone was not greater than that observed in the nontoxic gravid patients.

Acknowledgment

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REFERENCES

1. Simpson, S. A.; Tait, J. F.; Wettstein, A.; Néher, R.; von Euw, J.; Schindler, O., and Reichstein, T.: Konstitution des Aldosterons, des neuen Mineralocorticoids, *Experientia* **10**: 132, 1954.
2. Deming, Q. B., and Leutscher, J. A., Jr.: Bioassay of desoxycorticosterone-like material in urine, *Proc. Soc. Exper. Biol. & Med.* **73**: 171, 1950.
3. Chart, J. J.; Shipley, E. G., and Gordon, E. S.: Evidence for a sodium retaining factor in toxemia of pregnancy, *Proc. Soc. Exper. Biol. & Med.* **78**: 244, 1951.
4. Venning, E. H.; Simpson, G. A., and Singer, B.: Adrenocortical function in toxemia of pregnancy, *Am. J. Obst. & Gynec.* **67**: 542, 1954.
5. Gordon, E. S.; Chart, J. J.; Hagedorn, D., and Shipley, E.: Mechanism of sodium retention in preeclamptic toxemia, *Obst. & Gynec.* **4**: 39, 1954.
6. Venning, E. H., and Dyrenfurth, I.: Aldosterone excretion in pregnancy, *J. Clin. Endocrinol. & Metab.* **16**: 426, 1956.
7. Venning, E. H.; Dyrenfurth, I., and Giroud, C. J. P.: Aldosterone excretion in healthy persons, *J. Clin. Endocrinol. & Metab.* **16**: 1326, 1956.
8. Silber, R. H., and Porter, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923, 1954.
9. Hutchison, D. L.; Plentl, A. A., and Taylor, H. C., Jr.: The total body water and the water turnover in pregnancy studied with deuterium oxide as isotopic tracer, *J. Clin. Invest.* **33**: 235, 1954.
10. Tysoe, F. W., and Lowenstein, L.: Blood volume and hematologic studies in pregnancy and the puerperium, *Am. J. Obst. & Gynec.* **60**: 1187, 1950.
11. Caton, W. L.; Roby, C. C.; Reid, D. E., and Gibson, J. C.: Plasma volume and extravascular fluid volume during pregnancy and the puerperium, *Am. J. Obst. & Gynec.* **57**: 471, 1949.
12. Chesley, L. C.: A study of extracellular water changes in pregnancy, *Surg., Gynec. & Obst.* **26**: 589, 1943.
13. Taylor, H. C., Jr.; Warner, R. C., and Welsh, C. A.: Relationship of estrogens

and other placental hormones to sodium and potassium balance at end of pregnancy and in puerperium, *Am. J. Obst. & Gynec.* **38:** 748, 1939.

14. Gray, M. J., and Plentl, A. A.: The variations of the sodium space and the total exchangeable sodium during pregnancy, *J. Clin. Invest.* **33:** 347, 1954.
15. Venning, E. H.: Adrenal function in pregnancy, *Endocrinology* **39:** 203, 1946.
16. Tobian, L., Jr.: Cortical steroid excretion in edema of pregnancy, pre-eclampsia, and essential hypertension, *J. Clin. Endocrinol.* **9:** 319, 1948.
17. Bayliss, R. I. S.; Browne, J. C.; Round, B. P., and Steinbeck, A. W.: Plasma 17-hydroxycorticosteroids in pregnancy, *Lancet* **1:** 62, 1955.
18. Gemzell, C. A.: Blood levels of 17-hydroxycorticosteroids in normal pregnancy, *J. Clin. Endocrinol. & Metab.* **13:** 898, 1953.
19. Taylor, H. C., Jr.; Warner, R. C., and Welsh, C. A.: Relationship of the estrogens and progesterone to the edema of normal and toxic pregnancy, *Am. J. Obst.* **45:** 547, 1943.
20. Luetscher, J. A., Jr., and Axelrad, B. J.: Increased aldosterone output during sodium deprivation in normal men, *Proc. Soc. Exper. Biol. & Med.* **87:** 650, 1954.
21. Falbriard, A.; Muller, A. F.; Neher, R., and Mach, R. S.: Étude des variations de l'aldostéronurie sous l'effet de surcharges en potassium et de déperditions rénales et extrarenales de sel et d'eau, *Schweiz. med. Wchnschr.* **85:** 1218, 1955.
22. Liddle, G. W.; Bartter, F. C.; Duncan, L. E., Jr.; Barber, J. K., and Delea, C.: Mechanisms regulating aldosterone production in man, *J. Clin. Invest.* **34:** 949, 1955.
23. Beck, J. C.; Dyrenfurth, I.; Giroud, C. J. P., and Venning, E. H.: Observations on the regulatory mechanism of aldosterone secretion in man, *Arch. Int. Med.* **96:** 463, 1955.
24. Bartter, F. C.; Liddle, G. W.; Duncan, L. E., and Delea, C.: The role of extracellular fluid in the control of aldosterone secretion in man, *J. Clin. Invest.* **35:** 668, 1956.
25. Giroud, C. J., and Venning, E. H.: Unpublished data.
26. Mach, R. S.; Fabre, J.; Muller, A. F., and Neher, R.: Oedèmes par rétention de chlorure de sodium avec hyperaldostéronurie, *Schweiz. med. Wchnschr.* **85:** 1229, 1955.
27. De Courcy, C.; Gray, C. H., and Lunnon, J. B.: Adrenal cortical hormones in human placenta, *Nature* **170:** 494, 1952.
28. Johnson, R. H., and Haines, W. J.: Extraction of adrenal cortex hormone activity from placental tissue, *Science* **116:** 456, 1952.
29. Salhanick, H., Jones, J. E., and Berliner, D.: Sources of placental steroids, *Fed. Proc.* **15:** 160, 1956.
30. Jailer, J. W., and Knowlton, A. I.: Simulated adreno-cortical activity during pregnancy in an addisonian patient, *J. Clin. Invest.* **29:** 1430, 1950.
31. Opsahl, J. C., and Long, C. N. H.: Identification of ACTH in human placental tissue, *Yale J. Biol. & Med.* **24:** 199, 1951.

THE DISTURBANCE IN IODINE METABOLISM PRODUCED BY THYROID SURGERY

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IT IS generally held that the concentration of protein-bound radioiodine (PBI^{131}) in a sample of plasma taken forty-eight or seventy-two hours following administration of a tracer dose of I^{131} is a reliable index of thyroid function (1–6). It has been occasionally noted, however, that in patients successfully treated for hyperthyroidism the PBI^{131} tends to remain above the generally accepted level of normality and thus to indicate the presence of thyrotoxicosis in subjects who are euthyroid or even myxedematous in all clinical respects (7–9). During the past year several papers (10–13), including one from this laboratory (14), have appeared reporting more extensive results of routine radioiodine tests, and confirming that the PBI^{131} test and the "conversion ratio" may be frequently misleading in patients previously treated for thyroid disease by either subtotal thyroidectomy or irradiation with radioiodine. Parallel to the increase in the rate of appearance of PBI^{131} in the circulation, an increased rate of release of I^{131} by the thyroid could be observed in these patients, even if they were treated for cardiac disease and had never been thyrotoxic (14, 15). The obvious explanation was to relate the apparent increase in the turnover of I^{131} in the thyroid to the considerable reduction in the size of the thyroidal organic iodine pool brought about by these two forms of therapy—the I^{131} then being distributed in a smaller thyroidal iodine pool and a normal hormonal secretion rate corresponding to the secretion of a larger fraction of thyroidal I^{131}.

The present paper reports some results of a more detailed study of iodine metabolism in 4 patients who were previously subtotally thyroidectomized for nontoxic adenomatous goiter, and in 1 who had been operated upon for toxic goiter and at the time of study was suffering from a relapse. They have been compared with results of a similar study of iodine metabolism in 5 untreated thyrotoxic patients. In 3 of the patients treated by surgery it was observed that I^{131} was released by the thyroid, appeared in the plasma, and was removed from the plasma at two distinctly different rates. These

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observations led the authors to believe that the cells of the thyroid gland form (even under normal conditions) at least two iodinated compounds, presumably thyroxine and triiodothyronine, and that the latter is more readily secreted into, and more rapidly removed from, the circulation than thyroxine.

METHODS

The 10 subjects were hospitalized patients whose thyroid function had been established on the basis of a careful physical examination, case history, and results of various laboratory tests including those of the detailed I^{131} study. Two patients were males and 8 were females.

The radioiodine (I^{131}) was obtained from A.E.R.E., Harwell, as a carrier-free iodide solution. A dose of 100 microcuries (μc.) containing 10 micrograms (μg.) of stable iodide was given intravenously. An equal amount was placed in a glass model resembling a normal thyroid in size and shape, the model being immersed in a beaker filled with water. A third dose was diluted with tap water in such fashion as to attain a final concentration of 0.1 per cent of the dose in 10 ml. Urine was collected at daily intervals in bed pans which contained a drop of strong potassium iodide solution.

The I^{131} content of the thyroid was estimated with a collimated scintillation counter fitted with a $1\frac{1}{2}\times 1$-inch crystal of sodium iodide at a skin-crystal distance of 37 cm. A 1-mm. lead filter was employed to correct for weak scatter radiations. The thyroidal radioactivity was obtained by subtracting from the total neck activity the extrathyroidal activity which, measured during the third minute following injection, was assumed to decrease at a rate which was parallel to that of plasma I^{131} concentration (16). Most of the 10-ml. plasma and urine samples were measured in a flat-bottomed glass cup put on top of the crystal. With this arrangement, 1 μc. of I^{131} in 10 ml. registered about 3×10^5 counts per minute. Recently, a scintillation counter was used in which the sample, contained in a moulded polythene cup, surrounded a smaller crystal; this arrangement was less sensitive, but the counting rate:background ratio was more satisfactory.

Plasma protein-bound I^{131} concentration was determined after precipitation of the plasma proteins by dropwise addition of ice-cold trichloroacetic acid. The precipitate was washed twice with diluted TCA and then redissolved and made up to 10 ml. with caustic soda. In the latter part of the study it became our practice to check the results obtained with the precipitation method by simultaneous determination of plasma activity after the I^{131} present in the form of iodide was removed by passing the sample through a column of an anion exchange resin (Amberlite IRA 400). Results not agreeing within 5 per cent were discarded. At least 10,000 counts were made in all measurements, and all results were expressed as fractions of the administered dose. Details of the calculations are furnished in the Appendix.

RESULTS

The similarity between the results of routine I^{131} tests in patients subjected to thyroid surgery and in patients with thyrotoxicosis suggested that a comparison be made between the results of the more detailed studies of iodine metabolism carried out in both groups. The data obtained in 5 patients with moderate to severe thyrotoxicosis are presented in Table 1. In all cases iodine metabolism appeared to be adequately described by the

three-compartment model of Riggs (17), and calculations based on this model yielded values within the expected range. In all cases high "theoretical" uptakes and increased rates of turnover of organic iodine in the thyroid and in the extrathyroidal compartment could be observed. There appeared to be a significantly positive correlation between the rate constant of hormonal secretion and the "theoretical" uptake, and a significantly neg-

TABLE 1. TURNOVER AND DISTRIBUTION DATA CALCULATED FROM OBSERVED BEHAVIOR OF RADIOIODINE TRACER DOSE

Patient	U* Prop.	k_G	k_{GB}	$\bar{k}_B$	b_{ret}+ Prop./L.	C_E	C_t	G′	B′	V_B Liters
		Proportion per day				Liter per day		Proportion		
Patients with thyrotoxicosis										
STR	0.896	0.072	0.371	0.253	0.036	0.367	3.391	0.405	0.595	16.5
SEI	0.702	0.048	0.130	0.422	0.028	0.940	3.500	0.765	0.235	8.4
HIR	0.862	0.102	0.455	0.610	0.040	0.741	5.973	0.574	0.426	10.65
MOR	0.918	0.202	1.152	0.490	0.062	0.580	5.792	0.298	0.702	11.3
WIL	0.804	0.114	0.412	0.286	0.035	0.780	4.422	0.410	0.590	16.85
Patients previously treated by surgery										
KLI	0.425	0.016	0.026	0.134	0.0096	1.229	2.375	0.838	0.162	16.9
ZUP	0.630	0.075	0.173	0.196	0.056	0.589	1.767	0.530	0.470	8.4

* For explanation of symbols *see* list at beginning of Appendix.

ative correlation between this constant and the fraction of organic iodine stored in the thyroid. There was, however, no relation between the rate constants of hormonal secretion and organic iodine removal from the extrathyroidal compartment. The volume of the extrathyroidal organic iodine compartment was generally much smaller than the theoretical value of twenty-four liters assumed by Riggs (17) and the values observed in normal subjects by Hickey and Brownell (18), but more in line with the values observed in normal and in thyrotoxic cases by Berson and Yalow (19).

Additional information on the state of iodine metabolism would have been obtained if simultaneous determinations of either the plasma PBI^{127} level or the daily urinary I^{127} excretion had been made. However, the assumption that these patients, being on a normal hospital diet, had an average iodine intake of 150 μg. per day is probably not far from the truth. By introducing this value into the equations used for calculation of the turnover data, a further set of values could be calculated which is listed in Table 2. In all the thyrotoxic subjects the hormonal secretion rate thus calculated was much higher than the generally accepted normal value of about 100 μg. of iodine per day. The iodine content of the thyroid was considerably reduced. The calculated value of plasma PBI^{127} concentration

obtained by dividing the amount of extrathyroidal organic iodine by its distribution volume, was in all cases either equal to or higher than the accepted upper limit of 8.0 μg. per 100 ml.

These results would indicate that even in cases with considerably increased iodine turnover, the main pathways of iodine metabolism were adequately described by the three-compartment model, and that the introduction of the various assumptions mentioned did not do any serious

TABLE 2. APPROXIMATE VALUES OF HORMONAL SECRETION RATE, ORGANIC IODINE POOLS AND PLASMA PROTEIN-BOUND IODINE, CALCULATED FROM DATA OF TABLE 1 AND FROM ASSUMED VALUE OF IODINE INTAKE

Patient	H* μg./day	Q_G μg.	Q_B μg.	b μg./100 ml.
Patients with thyrotoxicosis				
STR	690	1870	2740	16.9
SFI	290	2200	680	8.0
HIR	580	1270	950	8.9
MOR	790	680	1610	14.2
WIL	440	1060	1520	9.0
Patients previously treated by surgery				
KLI	100	3960	770	4.6
ZUP	220	1260	1110	13.2

* For explanation of symbols *see* list at beginning of Appendix.

violence to the major facts. It is mainly for this reason that our data on thyrotoxic cases are presented here. However, in only 2 of the 5 patients previously subjected to thyroid surgery were observations on iodine metabolism obtained which were consistent with the three-compartment model (Tables 1 and 2):

One patient (KLI) had been thyroidectomized thirty-one years before. She was clinically euthyroid and the results of her routine I^{131} tests as well as of the other laboratory tests were well within the normal range. A normal amount of hormonal iodine was secreted, and the iodine content of her thyroid, though half the normal value, was the highest calculated in this series. The other patient (ZUP) had been operated upon about one and a half years before for toxic goiter, and at the time of study clearly had recurrent thyrotoxicosis. The observed turnover of the tracer dose and the data calculated therefrom were consistent with this diagnosis.

In the 3 other thyroidectomized patients the observations could not be

explained by the three-compartment model. They had all been operated upon for nontoxic goiter within one year prior to the present study and were either euthyroid or even slightly myxedematous, with basal metabolic rates ranging from −6 to +21 per cent. In these cases thyroidal release of organic I^{131} proceeded during the first few days at a rate which was several times faster than the release rate during the following days (Fig. 1). Correspondingly, I^{131} appeared in the plasma in protein-bound form also at two

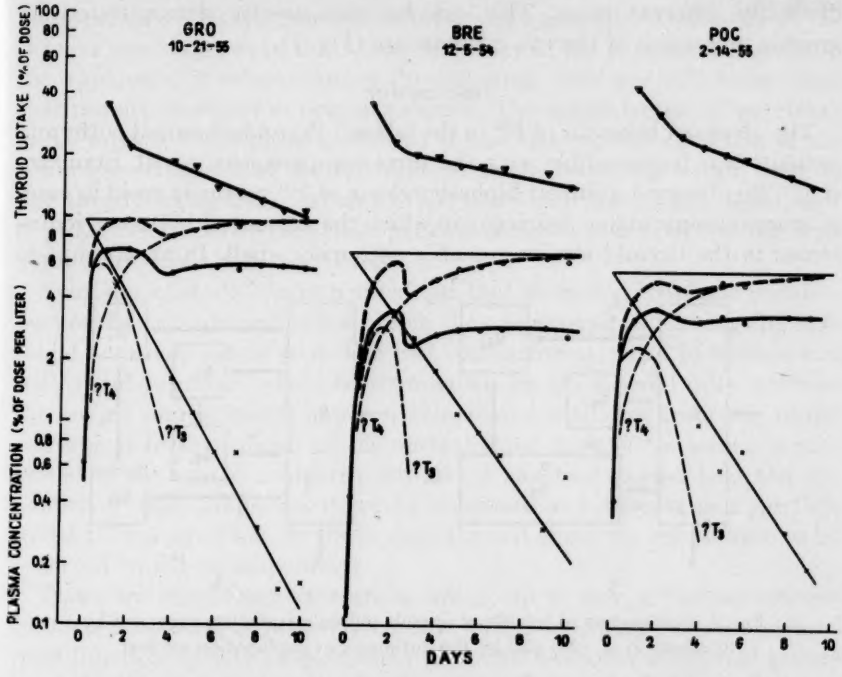

FIG. 1. Behavior of tracer dose in the thyroid and in the plasma in 3 patients previously treated by surgery for nontoxic goiter.

The shape of the curves of thyroidal I^{131} content (triangles) and plasma PBI^{131} (solid dots) suggested the thyroidal release of two iodinated compounds having different metabolic fates. The rate of appearance of the slow component, presumably thyroxine (T_4), was calculated by subtracting the concentration values expressed as a fraction of the dose retained (I_{ret}^{131}, circles) from the final equilibrium value and fitting a straight line to the obtained differences (crosses). Connecting this line with the equilibrium value at zero time permitted extrapolation of the T_4 build-up curve to time zero. Subtraction of the values thus obtained from the actually observed concentration values (circles) yielded a curve describing the behavior of the fast component, presumably labeled triiodothyronine (T_3).

different rates. In all cases there was a steep rise in plasma activity to very high levels, then (roughly corresponding to the change in the slope of thyroidal release) a fall and then an increase again. The observations were not extended for a period sufficiently long to observe the final slow decrease.

This type of I^{131} turnover is in sharp contrast to the monophasic curves of thyroidal release and plasma appearance seen in the thyrotoxic patients. Obviously, in these 3 thyroidectomized subjects hormonal I^{131} was not only released by the thyroid but also removed from the plasma at two distinctly different rates. This can be conveniently demonstrated by graphic separation of the two components (Fig. 1).

DISCUSSION

The observed behavior of I^{131} in the latter 3 thyroidectomized euthyroid patients was incompatible with the three-compartment model. Stanbury *et al.* (20) observed a similar biphasic release of I^{131} by the thyroid in cases of severe chronic iodine deficiency in which the amount of hormonal iodine stored in the thyroid was presumably extremely small. In an attempt to

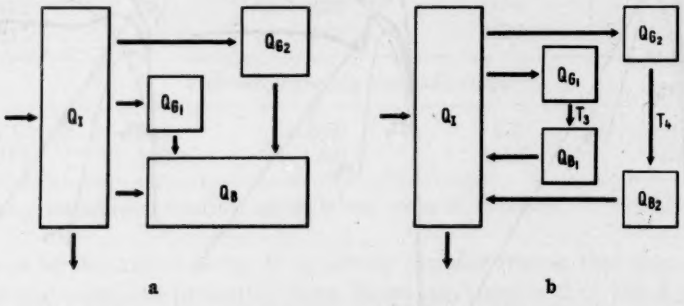

FIG. 2. Comparison of models of organic iodine metabolism proposed by Stanbury *et al.* (20) and by the authors. For explanation *see* text.

describe the kinetics of iodine metabolism in these cases more adequately, they designed an alternative model in which the iodine in the thyroid is considered to occupy two compartments of different magnitude and with different release rates (Fig. 2a). This model should be equally suitable for describing the biphasic thyroidal I^{131} release seen in our cases. However, in order to take into account that the hormonal I^{131} was not only secreted into but also removed from the circulation at two different rates, we had to choose an alternative model (Fig. 2b) in which the extrathyroidal hormonal iodine pool is divided into two further compartments; thus the present model is in fact a five-compartment model. To avoid the introduction of still another thyroidal compartment, the iodine present in the thyroid

in the form of inorganic iodide has been assigned in both models to the iodide pool of the body.

As it would be almost impossible to set up and to solve the differential equations which would adequately represent the fate of labeled iodine in this model, no attempt was made to calculate the various turnover data as has been done on the basis of the three-compartment model in the other cases. However, on the basis of clinical evidence, it could be presumed that these patients produced not more than a normal amount of thyroid hormone per day. The rate constant of the initial apparent I^{131} release from the thyroid was higher than that observed in any of the thyrotoxic cases, and the apparent I^{131} release during the following days was still faster than that usually observed in normal subjects. The concentration of extrathyroidal organic I^{131} in plasma, whether it was expressed as a fraction of the administered dose or of the dose retained in the body, was within the first day already higher than that seen in the thyrotoxic cases. Thus the amount of preformed hormone that these patients could store in their thyroid remnants must have been very small.

Stanbury *et al.* (20) have pointed out that there is a histologic counterpart of the two compartments, which they considered as forming the thyroidal hormonal iodine pool. The first compartment, small in volume and with rapid turnover, would be represented by the thyroid cells; whereas the second compartment, larger in volume and with slow turnover, would correspond to the colloid. In the normal gland most of the iodine is contained in the colloid compartment, and it can be expected that the dynamics of this compartment would dominate any observations on thyroidal I^{131} release. Thus, in the normal thyroid gland the release rate to be observed would be monophasic.

There are three conditions under which, up to now, a biphasic release rate has been observed—severe chronic iodine deficiency (18), metastases from functioning thyroid carcinoma (21), and remnants of thyroid glands left behind at subtotal thyroidectomy. Histologically, they have in common that the colloid/cell ratio is greatly altered in favor of the cells. The chronic iodine-deficient goiter in its later stages is poor in colloid; functioning thyroid carcinoma is characterized by great numbers of thyroid cells frequently arranged in papillae or in compact masses; the thyroid remnant immediately after operation starts to regrow by forming firstly new cells and only later on (when hormone production exceeds the daily needs) by forming additional colloid for hormone storage. Thus, in conditions characterized by a significant reduction of the colloid/cell ratio the I^{131} turnover in the colloid becomes less dominating and the separate release of I^{131} by the cells can be observed.

Though this hypothesis should serve to explain why in our cases I^{131} was

released by the thyroid at two different rates, it offers no explanation for the fact that I^{131} was also removed from the plasma at two different rates. To take this phenomenon into account we have to go one step further in our speculations and assume that the thyroid secretes two different iodinated compounds having different fates in the body.

There are three independent observations which at once come into mind: 1) from experiments with labeled triiodothyronine, it is known that this compound disappears from plasma about four times faster than thyroxine (22–24); 2) following a therapeutic dose of I^{131}, labeled triiodothyronine may appear in the circulation within one hour and prior to labeled thyroxine (25); and 3) the final equilibrium value of the plasma I_{ret}^{131} concentrations is approximately the same for both distribution rates (Fig. 1) although the distribution volume of triiodothyronine is probably considerably larger than that of thyroxine (26).

The most logical hypothesis embodying these observations would then be that not only thyroxine but also triiodothyronine is formed by the thyroid cells and secreted into the circulation. This process may involve a transient binding to the colloid protein but in a more loose form than that of thyroxine, similar to the less strong bondage of triiodothyronine to plasma protein (27). In terms of rates, more triiodothyronine would be secreted by the thyroid per unit time than thyroxine, but as it is distributed in a larger volume and is metabolized at a faster rate than thyroxine, its concentration in the plasma remains small.

This hypothesis would imply that triiodothyronine is not only a product of peripheral deiodination of thyroxine, as has been originally suggested (28, 29); it is compatible with the theory of Roche and his colleagues that there is an additional mechanism in the thyroid by which triiodothyronine is formed by coupling one molecule of monoiodotyrosine with one molecule of diiodotyrosine (30, 31).

As a further bit of evidence supporting this hypothesis, results of some preliminary experiments now in progress may be mentioned in which the observations of Benua *et al.* (25) were repeated and extended to patients previously subjected to thyroid surgery. Following administration of between 4 and 7 mc. of I^{131}, the butanol extracts of plasma samples taken one and four hours later were subjected to paper chromatography and the strips subsequently analyzed for distribution of radioactivity. It was found in thyroidectomized patients that in both samples more than 90 per cent of the PBI^{131} consisted of labeled triiodothyronine, and that as late as four hours following administration of the dose only traces of labeled thyroxine were present (32). This result would be in accord with the *in vivo* observations of thyroidal I^{131} release and would clearly demonstrate that triiodo-

thyronine must have been secreted into the circulation separately and prior to thyroxine.

It was previously stated that soon after operation the colloid/cell ratio is reduced because of the initial predominance of cell regrowth. However, with increasing time after operation one would expect the colloid/cell ratio to return to normal. If this be true, then the effects of subtotal thyroidectomy on the metabolism of a dose of I^{131} should become less marked the longer the period from the time of operation to the time of the I^{131} study. In Patient KLI, who had been thyroidectomized thirty-one years previously, it was noted that her iodine metabolism was almost normal. In Figure 3 the values of the plasma I^{131} concentration at forty-eight hours have been correlated with the time which had elapsed since operation in 55 patients who had been operated upon within ten years prior to the present study, mostly because of nontoxic goiter. They were all in a clinically euthyroid state. As most of the administered dose still in the form of iodide had at forty-eight hours already been excreted (none of the patients showed evidence of myxedema or impaired renal function), this value was representative of the 48-hour PBI^{131} level. The patients were selected at random, so there were large variations as to the case history, the extent of surgical removal, the average iodine intake following operation, the body weight and associated factors such as the volume of the PBI^{131} distribution space. Since the PBI^{131} level is inversely proportional to the iodine content of the gland (17) it appeared logical to plot versus time the logarithm of the plasma I^{131} concentration (Fig. 3). It will be seen that with increasing time after operation, elevation of the plasma I^{131} level became less marked; the negative correlation was highly significant with a *t*-value of 3.72 and a probability of less than 0.1 per cent that this result could have arisen by chance. On the average, it appeared to require about seven years for the plasma I^{131} concentration to return to a value below 0.5 per cent per liter, which can be taken as the upper limit of normal results. This time, however, should be appreciably shorter

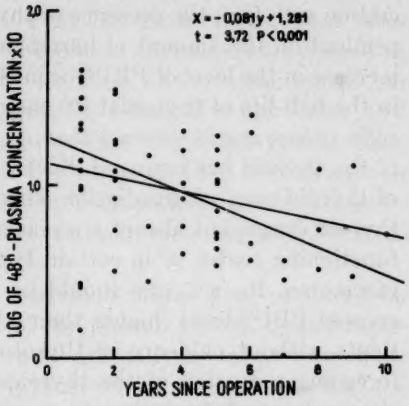

FIG. 3. Relation observed in 55 patients between log of plasma I^{131} concentration at forty-eight hours after administration of tracer dose and length of time in years since thyroid surgery.

in patients living in a region of chronic iodine abundance.

This observation demonstrates how large a percentage of euthyroid patients previously treated by thyroid surgery will have a PBI^{131} level suggestive of that in thyrotoxicosis. All the so-called "organic" I^{131} function tests, though usually correctly recording an increase in the hormonal secretion rate (*i.e.*, the presence of thyrotoxicosis), appear to be strongly dependent on the amount of hormonal iodine stored in the thyroid (14); no increase in the level of PBI^{131} or in the "conversion ratio" and no reduction in the half-life of thyroidal I^{131} should be taken as indicative of thyrotoxicosis unless one is assured that no gross reduction of the iodine content of the thyroid has occurred. Such a reduction may not only be the result of thyroid surgery, irradiation with I^{131} or prolonged treatment with antithyroid drugs, but also of a replacement of normal thyroid tissue by nonfunctioning tissue, as in certain types of congenital goiter and of thyroid carcinoma. In fact, one should be alarmed by the observation of an increased PBI^{131} level (higher than 0.5 per cent of the dose per liter) in patients without evidence of thyrotoxicosis and without any history of a foregoing reduction of the thyroidal hormonal pool, and should begin a thorough search for early symptoms of thyroid carcinoma.

Since the so-called "inorganic" I^{131} function tests (thyroidal clearance, early uptake, urinary excretion) are, to a certain extent, independent of the amount of stored hormone, the diagnosis of recurrent thyrotoxicosis should be made possible by the use of one of these tests. This, however, is true only for patients living in a region of iodine abundance. In regions of chronic iodine deficiency, like Austria and many other countries, values for thyroidal clearance, thyroidal uptake, and urinary excretion in euthyroid patients fall well within that range which, under normal conditions, would be taken to indicate the presence of thyrotoxicosis. Any one of the "organic" I^{131} tests (PBI^{131} concentration at forty-eight hours, "conversion ratio," $48^h/2^h$-ratio, effective thyroidal half-life) has to be carried out in addition to "inorganic" tests in order to permit differentiation between the high uptakes (or low excretions) of thyrotoxicosis and of nontoxic iodine-deficient goiter (33, 34). However, in previously treated patients the "organic" tests may also yield results indistinguishable from those found in true thyrotoxicosis. In this event there remains no means, on the basis of *in vivo* I^{131} function tests alone, for making a definite diagnosis of the functional status of the thyroid remnants in these patients.

SUMMARY

1. In 5 patients subjected to thyroid surgery the metabolism of iodine was studied by means of radioiodine techniques, and compared with that observed in 5 thyrotoxic patients.

2. Whereas in the thyrotoxic cases and in 2 of the thyroidectomized sub-

jects iodine metabolism could be adequately described by a three-compartment model, the development of a five-compartment model was required to explain the observations made in the other 3 euthyroid patients who had been operated upon for nontoxic goiter within the previous year. In these 3 cases, thyroidal radioiodine and protein-bound plasma radioiodine were removed from their respective pools at two distinctly different rates.

3. It is postulated that the observed biphasic thyroidal and plasma removal of radioiodine is caused by the secretion and distribution of two different iodinated compounds, presumably triiodothyronine and thyroxine. It is assumed that formation and secretion of triiodothyronine takes place also in the normal gland but that this compound becomes detectable to *in vivo* radioiodine techniques only in conditions which are characterized by a reduced thyroidal hormonal iodine pool and a decreased colloid/cell ratio altered in favor of the cells.

4. The reduction in the thyroidal hormonal iodine pool produced by thyroid surgery leads to an elevation of the plasma protein-bound radioiodine concentration to levels which would normally be taken to indicate the presence of thyrotoxicosis. Observations on 55 thyroidectomized patients have shown that, following operation, this effect gradually diminishes with time.

REFERENCES

1. Williams, R. H.; Jaffe, H., and Bernstein, B.: Comparison of the distribution of radioactive iodine in serum and urine in different levels of thyroid function, *J. Clin. Invest.* **28**: 1222, 1949.
2. Freedberg, A. S.; Ureles, A., and Hertz, S.: The serum level of protein-bound radioactive iodine (I^{131}) in the diagnosis of hyperthyroidism, *Proc. Soc. Exper. Biol. & Med.* **70**: 679, 1949.
3. Silver, S., and Fieber, M. H.: Blood levels of I^{131} after tracer doses in the diagnosis of hyperthyroidism, *Proc. Soc. Exper. Biol. & Med.* **75**: 570, 1950.
4. Silver, S.; Fieber, M. H., and Yohalem, S. B.: Blood levels after tracer doses of radioactive iodine in the diagnosis of thyroid disorders, *Am. J. Med.* **13**: 725, 1952.
5. Goodwin, J. F.; MacGregor, A. G.; Miller, H., and Wayne, E. J.: The use of radioactive iodine in the assessment of thyroid function, *Quart. J. Med.* **20**: 353, 1951.
6. Horst, W.: Die diagnostische und therapeutische Anwendung des Radiojodids (131 J), *Fortschr. Geb. Röntgenstrahlen* **77**: 567, 1952.
7. Blom, P. S., and Terpstra, J.: High PBI^{131} concentration in blood of patients with myxedema: preliminary report, *J. Clin. Endocrinol. & Metab.* **13**: 989, 1953.
8. Wayne, E. J.: The diagnosis of thyrotoxicosis, *Brit. M. J.* **1**: 411, 1954.
9. Ingbar, S. H.; Freinkel, N.; Hoeprich, P. D., and Athens, J. W.: The concentration and significance of the butanol-extractable I^{131} of serum in patients with diverse states of thyroidal function, *J. Clin. Invest.* **33**: 388, 1954.
10. Paley, K. R.; Sobel, E. S., and Yalow, R. S.: A comparison of the thyroidal plasma I^{131} clearance and the plasma protein-bound I^{131} tests for the diagnosis of hyperthyroidism, *J. Clin. Endocrinol. & Metab.* **15**: 995, 1955.
11. Silver, S.; Yohalem, S. B., and Newburger, R. A.: Pitfalls in diagnostic use of radioactive iodine, *J.A.M.A.* **159**: 1, 1955.

12. LINDEBOOM, G. A.; HOOGENDIJK-VAN DORT, Tj. E., and DE JONG, J.: Blood levels of I^{131} after tracer doses in patients with thyrotoxicosis treated with radio-iodine, *Acta med. scandinav.* **40**: 487, 1955.
13. NODINE, J. H.; PERLOFF, W. H.; SOPP, T. E.; FERRANDIS, R. N., and DE ALBUQUERQUE, D.: The thyrotoxic remnant, *Am. J. M. Sc.* **230**: 397, 1955.
14. VETTER, H.: Die Diagnostik der Schilddrüsen-Erkrankungen mit radioaktivem Jod, *Ergebn. inn. Med. u. Kinderheilk.* (N.F.) **6**: 695, 1955.
15. FREEDBERG, A. S.; CHAMOVITZ, D. L., and KURLAND, G. S.: Thyroid function in normal and pathological states as revealed by radioactive iodine studies. I. Thyroid I^{131} uptake and turnover in euthyroid, hyperthyroid and hypothyroid subjects, *Metabolism* **1**: 26, 1952.
16. FELLINGER, K.; HÖFER, R., and VETTER, H.: Salivary and thyroidal radioiodide clearances of plasma in various states of thyroid function, *J. Clin. Endocrinol. & Metab.* **16**: 449, 1956.
17. RIGGS, D. S.: Quantitative aspects of iodine metabolism, *Pharmacol. Rev.* **4**: 284, 1952.
18. HICKEY, F. C., and BROWNELL, G. L.: Dynamic analysis of iodine metabolism in 4 normal subjects, *J. Clin. Endocrinol. & Metab.* **14**: 1423, 1954.
19. BERSON, S. A., and YALOW, R. S.: Quantitative aspects of iodine metabolism. The exchangeable organic iodine pool, and the rates of thyroidal secretion, peripheral degradation and fecal excretion of endogenously synthesized organically bound iodine, *J. Clin. Invest.* **33**: 1533, 1954.
20. STANBURY, J. B.; BROWNELL, G. L.; RIGGS, D. S.; PERINETTI, H.; ITOIZ, J., and DEL CASTILLO, E. B.: Endemic Goiter: The Adaptation of Man to Iodine Deficiency. Cambridge, Mass., Harvard Univ. Press, 1954.
21. POCHIN, E. E.: The speed of iodine metabolism, Paper A/CONF.8/P/769, read before the International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1955.
22. RAWSON, R. W.; RALL, J. E.; PEARSON, O. H.; ROBBINS, J.; POPPELL, H. F., and WEST, C. D.: *l*-Triiodothyronine versus *l*-thyroxine. A comparison of their metabolic effects in human myxedema, *Am. J. M. Sc.* **226**: 405, 1953.
23. VAN ARSDEL, P., JR.; HOGNESS, J. R.; WILLIAMS, R. H., and ELGEE, N.: Comparative distribution and fate of I^{131}-labeled thyroxine and triiodothyronine, *Endocrinology* **55**: 332, 1954.
24. STERLING, K.; LASHOF, J. C., and MAN, E. B.: Disappearance from serum of I^{131}-labeled *l*-thyroxine and *l*-triiodothyronine in euthyroid subjects, *J. Clin. Invest.* **33**: 1031, 1954.
25. BENUA, R. S.; DOBYNS, B. M., and NINMER, A.: Triiodothyronine in the serum of patients treated with radioactive iodine, *J. Clin. Endocrinol. & Metab.* **15**: 1367, 1955.
26. POCHIN, E. E.: Personal communication.
27. DEISS, W. P.; ALBRIGHT, E. C., and LARSON, F. C.: Comparison of in vitro serum protein binding of thyroxine and triiodothyronine, *Proc. Soc. Exper. Biol. & Med.* **84**: 513, 1953,
28. GROSS, J., and PITT-RIVERS, R.: 3:5:3′-Triiodothyronine. 2. Physiological activity. *Biochem. J.* **53**: 652, 1953.
29. GROSS, J.: Thyroid hormones, *Brit. Med. Bull.* **10**: 218, 1954.

30. Roche, J.; Michel, R.; Lissitzky, S., and Michel, O.: Sur la formation d'iodures à partir de la diiodotyrosine dans le corps thyroïde et sur leur réutilisation, *Compt. rend. Acad. Sc.* (Paris) **232**: 2148, 1951.
31. Roche, J., and Michel, R.: Nature, biosynthesis and metabolism of thyroid hormones, *Physiol. Rev.* **35**: 583, 1955.
32. Vetter, H., and Wüstinger, G.: Unpublished observations.
33. Fellinger, K.; Mannheimer, E., and Vetter, H.: Zur Kenntnis der pathophysiologischen Grundlagen der Diagnostik und Therapie der Schilddrüsenerkrankungen mit radioaktivem Jod, *Strahlentherapie* **33**: 81, 1955.
34. Fellinger, K.; Mannheimer, E., and Vetter, H.: Der Radiojod-Plasmatest, *Wien. Ztschr. inn. Med.* **34**: 359, 1953.
35. Brenner, O.; Black, A. B., and Gaddie, R.: Estimation of the rate of thyroid hormone secretion in man, *Clin. Sc.* **13**: 441, 1954.
36. Brownell, G. L.: Analysis of techniques for the determination of thyroid function with radioiodine, *J. Clin. Endocrinol.* **11**: 1095, 1951.

APPENDIX

List of symbols used in calculations and tables, their units of measurement and definitions

Symbol	Unit	Definition
I		Compartment of inorganic iodide.
G		Compartment of thyroidal organic iodine.
B		Compartment of extrathyroidal organic iodine.
Q_I	μg.	Quantity of iodide in I.
Q_G		Quantity of organic iodine in G.
Q_B		Quantity of organic iodine in B.
G′	proportion	Fraction of total organic iodine in G.
B′		Fraction of total organic iodine in B.
U		"Theoretical" uptake. Fraction of Q_I accumulated by the thyroid.
V_B	liter	Volume of compartment B.
b	μg./100 ml.	Concentration of protein-bound I^{127} in plasma.
b^+	proportion per liter	Concentration of protein-bound I^{131} in plasma.
b^+_{ret}		Concentration of protein-bound I^{131} in plasma, expressed as fraction of the dose retained in the body.
In	μg./day	Daily intake of iodide.
H		Rate of secretion of organic iodine by the thyroid.
E		Rate of urinary excretion of iodide.
F		Rate of fecal excretion of organic iodine.

Symbol	Unit	Definition
k_{GB}	proportion per day	Rate constant of secretion of organic iodine by the thyroid.
k_G		Rate constant of "apparent" secretion of organic radioiodine by the thyroid.
k_{B_1}		Rate constant of removal of organic iodine from compartment B, calculated from build-up curve of b^+ in the plasma.
k_{B_2}		Rate constant of removal of organic iodine from compartment B, calculated from "apparent" organic radioiodine clearance of plasma.
$\bar{k}_B$		Mean of k_{B_1} and k_{B_2}.
C_G	liter per day	"Apparent" thyroidal organic I^{131} clearance of plasma.
C_E		"Apparent" renal organic I^{131} clearance of plasma.
C_F		Fecal organic I^{131} clearance of plasma.
C_t		Total "apparent" organic I^{131} clearance of plasma.
E^+	proportion per day	Fraction of dose excreted per day by the kidneys at equilibrium distribution of dose.

Calculations

The calculations of turnover rates and volumes of distribution of iodine in the thyroid and in the body have been based on the following assumptions and simplifications:

1. That after equilibrium distribution of the injected tracer dose within the iodide spaces of the body and the thyroid is established, the radioiodide is handled in the same manner as the stable iodide already present in these spaces. Since this study was mainly concerned with the metabolism of organic I^{131}, any delay in the attainment of iodide^{131} equilibrium was not likely to interfere seriously with the interpretation of observations extended over a period of several days.
2. That the daily excretion of organic iodine via the feces is 10 per cent of the amount secreted daily by the thyroid. Judging from published observations of fecal I^{131} excretion (19, 35), this value is probably of the correct order of magnitude, though there may occur large individual variations. All urinary radioactivity was assumed to be present in the form of iodide.
3. That the subjects studied were in a state of iodine equilibrium so that the iodide intake balanced the loss of iodine from the body, and the quantity of iodide collected by the thyroid was equal to the quantity of iodine secreted by the gland in organic form. It should be noted, however, that such a steady state is hardly ever reached, and that this assumption is wholly unjustified in cases of severe thyrotoxicosis in which the daily fecal excretion of iodine exceeds the daily intake of iodide and, therefore, the iodine balance of the body is negative, resulting in a progressive depletion of the iodine stores of the body.

Most of the calculations described in this paper are based on the three-compartment model of iodine metabolism developed by Riggs (17). In this model all the iodine of the

body is divided into three separate compartments: 1) the iodide compartment, I, which contains the inorganic iodide in the body, including the iodide in the secretions of the alimentary tract, and that iodide accumulated in the thyroid that has not yet been bound to tyrosine; 2) the compartment of organic iodine in the thyroid, G, which contains all the organic compounds of iodine present in the thyroid; and 3) the compartment of extrathyroidal organic iodine, B, which contains the organic iodine compounds and their degradation products outside the thyroid, including those undergoing entero-hepatic circulation.

In a subject in a steady state of iodine equilibrium, the amount of iodide daily ingested with food and water and adsorbed by the intestines, In, will be equal to the amount of iodine lost daily from the body via urine and feces, E and F. Thus:

$$In = E + F \qquad [1]$$

Since it is assumed that 10 per cent of the hormonal secretion rate of the thyroid, H, is excreted into the feces:

$$F = 0.1 . H \qquad [2]$$

Under equilibrium conditions, the amount of organic iodine secreted daily by the thyroid, H, will be equal to the amount of iodide daily accumulated by the thyroid. This amount will be a fraction, U, of the amount of iodide present in the body and, if a steady state obtain, will likewise be a fraction of all the iodide entering the iodide compartment, I. The iodide entering is the sum of the iodide intake, In, and of the iodide recirculated into the iodide pool from degradation of organic iodine in the peripheral tissues, H − F. Thus:

$$H = U . (In + H - F) \qquad [3$$

or

$$H = U . (In + 0.9 . H) \qquad [4]$$

Since the thyroid does not distinguish between radioactive and stable iodide, this proportion, U, is also that fraction of the administered dose distributed throughout the iodide compartment which is accumulated by the gland. In the present study, the I^{131} content of the thyroid was determined daily, starting twenty-four hours after administration of the tracer dose. At this time, part of the I^{131} accumulated will already have left the gland in the form of organic I^{131}. Therefore, the thyroidal uptake observed at twenty-four hours will always be less than the "theoretical" uptake, *i.e.*, that uptake which would be observed if no hormonal secretion took place. However, by successive daily estimations of the I^{131} content of the gland the rate of release of I^{131} from the thyroid can be determined, and a plot of the observed values on semi-log paper shows that a constant proportion of the I^{131} content of the gland is secreted daily. The value of the "theoretical" uptake, U, is arrived at by extrapolating back to time zero the straight line best fitting the daily values for thyroidal I^{131} content (Fig. 4).

Since the loss of labeled iodine from the gland is an exponential function, the biologic half-life of thyroidal I^{131} can be calculated and the rate constant of this loss can be obtained by dividing 0.693 by the half-life in days. Since part of the labeled iodine which is secreted by the gland and degraded in the peripheral tissues is recirculated into the iodide pool and re-utilized by the thyroid, the observed rate of loss of I^{131} from the gland will not reflect the rate of hormonal secretion; it may be termed the "apparent" rate of thyroidal iodine release and its rate constant, k_G, will always be smaller than the constant of the hormonal secretion rate, k_{GB}.

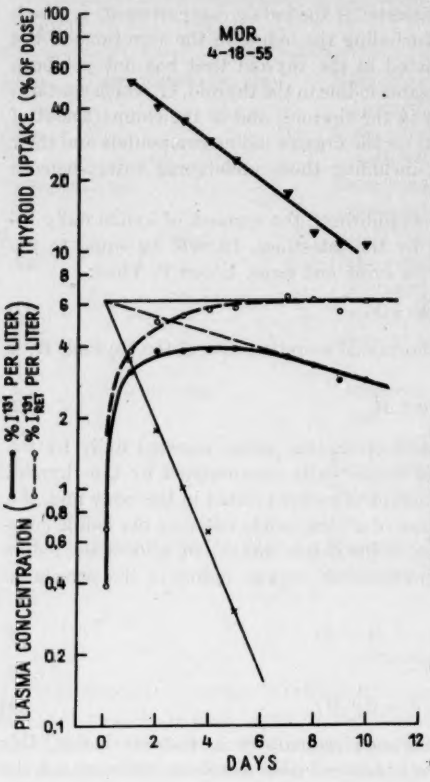

FIG. 4. Behavior of tracer dose in the thyroid and in the plasma in a case of severe thyrotoxicosis.

A semi-log plot of the values of thyroidal I^{131} content permits extrapolation to "theoretical" uptake at zero time. Rate of build-up of labeled PBI is calculated from line fitting the crosses which are found by subtracting the actual PBI^{131} values from dashed line connecting decreasing PBI^{131} values at equilibrium with final equilibrium value of PBI_{ret}^{131} per liter distribution volume (6.2 per cent of dose per liter).

If the radioiodide derived from hormonal degradation were not re-utilized by the thyroid, the hormonal secretion rate, H, would equal that fraction of the daily intake which is accumulated by the gland, U.In. In fact, the "apparent" rate constant of I^{131} release is not proportional to U.In, but rather to U.(In+H−F). Therefore:

$$\frac{k_G}{U.In} = \frac{k_{GB}}{U.(In + H - F)} \qquad [5]$$

or

$$\frac{k_G}{In} = \frac{k_{GB}}{In + 0.9.H} \qquad [6]$$

or

$$k_{GB} = k_G.\left(1 + 0.9.\frac{H}{In}\right). \qquad [7]$$

Solving Equation 4 for $\frac{H}{In}$:

$$U.In = H - U.\,0.9.H = H.(1 - 0.9.U)$$

or

$$\frac{H}{In} = \frac{U}{1 - 0.9.U}. \qquad [8]$$

Substituting in Equation 7:

$$k_{GB} = k_G.\left(1 + \frac{0.9.U}{1 - 0.9.U}\right) = \frac{k_G}{1 - 0.9.U}. \qquad [9]$$

Under equilibrium conditions, the amount of organic iodine leaving the thyroid and entering the compartment of extrathyroidal organic iodine daily will equal the amount of iodine leaving this compartment daily through peripheral degradation and fecal

excretion. Thus, by definition:

$$H = Q_G . k_{GB} = Q_B . k_B, \qquad [10]$$

where Q_G is the amount of organic iodine in G, Q_B the amount of organic iodine in B, and k_B the sum of the rate constants of peripheral degradation and fecal excretion, respectively. Due to the complexities involved in a rigorous mathematical description of the distribution of the tracer dose throughout the organic iodine pools, this rate constant k_B is one of the most difficult constants to measure. Brownell (36) has shown that a crude estimate can be obtained from the calculated half-time of build-up of hormonal I^{131} in plasma; as long as k_B is small compared to k_G, this rate of appearance is governed mainly by k_B. The value of k_B can be estimated graphically by subtracting the actual plasma concentrations of PBI^{131} plotted on semi-log paper, from the straight line obtained by extrapolation of the decreasing part of the plasma concentration curve.

Though it would appear logical that after equilibrium is established, I^{131} is removed from the thyroidal and extrathyroidal compartments at identical rates with the constant k_G, the authors were unable to find this hypothesis to be in accord with the actual results. In most cases of increased iodine turnover the rate of decrease of plasma radioactivity was observed to be smaller than the rate of decrease of thyroidal radioactivity. However, since the value which is arrived at by extrapolation to time zero is the theoretical plasma concentration which would be reached if none of the hormonal I^{131} left the organic iodine pool, this value, and therefore the slope of the extrapolated straight line, can be estimated by a modification of the method of calculation used by Berson and Yalow (19):

This method is based on the simplifying assumption that as soon as the fraction of the tracer dose accumulated by the thyroid is distributed between the thyroidal and extrathyroidal organic iodine pools, the fraction of the dose remaining in the body in the form of iodide is negligible. The sum of the fraction of the dose retained in the thyroid, G′, and the fraction retained in the extrathyroidal pool, B′, will then be unity:

$$G' + B' = 1. \qquad [11]$$

To evaluate the fraction of the dose remaining in the body, the fraction lost from the body must be known; it will be the sum of the cumulative daily urinary and fecal I^{131} excretions. The urinary excretion can easily be measured; the fecal excretion, which was not measured in the present study, can be approximated if it is again assumed that the organic iodine excretion rate amounts to 10 per cent of the thyroidal secretion rate, and if the time-lag between the onset of urinary and fecal I^{131} excretion is neglected.

The iodide derived from degradation of extrathyroidal organic I^{131} is either re-accumulated by the thyroid or excreted into the urine. As soon as equilibrium of the dose retained in the organic iodine pools is attained, the urinary radioiodide will represent clearance of metabolized plasma organic radioiodine. Thus, the "apparent" renal clearance of organic I^{131} can be estimated by dividing the amount of I^{131} excreted via the kidneys per unit time, E^+, by the PBI^{131} plasma concentration, b^+ :

$$C_E = \frac{E^+}{b^+} . \qquad [12]$$

The iodide returned to the iodide pool from peripheral degradation will be handled in the same manner as administered iodide. Therefore, the relation between the "apparent" thyroidal organic iodine clearance and the "apparent" renal organic iodine clearance will be the same as the relation between the fractions of the administered dose accumulated by the thyroid and excreted into the urine, respectively. Hence:

$$\frac{C_G}{C_E} = \frac{U}{1 - U} . \qquad [13]$$

The volume of organic iodine space which is cleared through peripheral breakdown per day, is then ($C_G + C_E$). Since under equilibrium conditions, the fecal clearance of organic I^{131} will amount to 10 per cent of the total clearance, C_t:

$$C_F = 0.1.(C_E + C_F + C_G) = \frac{C_E + C_G}{9} . \qquad [14]$$

The sum of the fractions of the dose lost from the body via urine and feces can now be calculated by multiplying the daily urinary excretions by the factor $(C_E+C_F)/C_E$, and the fraction of the dose retained in the body at any time by subtracting from the administered dose the cumulative urinary and fecal I^{131} excretions.

If the actual values of PBI^{131} are now divided by the fraction of the dose retained in the body, the resulting values expressed as concentrations per liter distribution volume approach an asymptotic value at distribution equilibrium. The slope of the straight line connecting this value, b_{ret}^+, at zero time with the actual plasma concentrations at distribution equilibrium (Fig. 4) permits a crude estimate of the rate by which organic iodine is removed from the extrathyroidal compartment B; its constant may be termed k_{B_I}.

It should be noted, however, that this method of estimating k_B from the rate of build-up of labeled hormonal iodine in plasma is theoretically valid only if k_B is considerably smaller than k_G. As this is neither the case in thyrotoxic nor in thyroidectomized subjects, the value of k_B estimated in this way might be considerably in error. A further method of estimating k_B was, therefore, needed:

Under equilibrium conditions, the dose retained in the body is distributed between the thyroidal and extrathyroidal organic iodine compartments as the respective amounts of stable iodine contained in these compartments. Hence:

$$\frac{G'}{B'} = \frac{Q_G}{Q_B} . \qquad [15]$$

From Equation 10 and Equation 11:

$$\frac{G'}{B'} = \frac{k_B}{k_{GB}} = \frac{1 - B'}{B'} \qquad [16]$$

or

$$k_B = k_{GB} \cdot \frac{1 - B'}{B'} . \qquad [17]$$

According to the definition for b_{ret}^+:

$$b_{ret}^+ = \frac{B'}{V_B} , \qquad [18]$$

where V_B is the volume of the extrathyroidal organic iodine compartment. A third equation is obtained from the definition that the rate constant of excretion is the clearance divided by the cleared volume:

$$k_B = \frac{C_t}{V_B} . \qquad [19]$$

Introducing Equation 18 and Equation 19 into Equation 17:

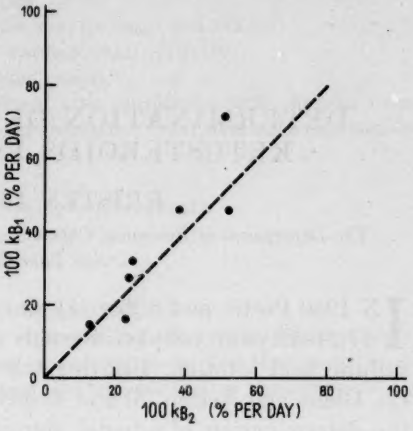

FIG. 5. Comparison of rates of removal of hormonal iodine from extrathyroidal pool, calculated either from rate of build-up of PBI^{131} in plasma (k_{B_1}) or from "apparent" organic I^{131} clearance of plasma (k_{B_2}). The dashed line represents a 1:1 relation.

$$k_B = k_{GB} \cdot \frac{1 - \dfrac{C_t \cdot b_{ret}^+}{\dfrac{C_t}{\bar{k}_B}}}{\dfrac{C_t \cdot b_{ret}^+}{\dfrac{C_t}{\bar{k}_B}}} = \frac{k_{GB} \cdot C_t \cdot b_{ret}^+}{k_{GB} - C_t \cdot b_{ret}^+} \qquad [20]$$

Since these three terms are known, k_B can be calculated by this second method and may be termed k_{B_2}.

It may be seen in Figure 5 that in the 7 subjects in whom both calculations could be performed, both values agreed within ± 20 per cent of their average. This average value, $\bar{k}_B$, was then used to calculate the volume V_B and the relative sizes of the compartments G and B:

$$V_B = \frac{C_t}{\bar{k}_B} \qquad [19a]$$

and

$$B' = b_{ret}^+ \cdot \frac{C_t}{\bar{k}_B} . \qquad [18a]$$

DETERMINATION OF 17,21-DIHYDROXY-20-KETOSTEROIDS IN BLOOD PLASMA*

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IN 1950 Porter and Silber (1) observed that a color reaction occurred if 17,21-dihydroxy-20-ketosteroids were exposed to a phenylhydrazine-sulfuric acid solution. Utilizing this color reaction, Nelson and Samuels (2), Glenn and Nelson (3) and Reddy *et al.* (4) have published methods for the determination of adrenal steroids having a 17,21-dihydroxy-20-keto configuration. Since then, numerous publications have appeared dealing with adrenocortical physiology and pathology, using levels of 17-hydroxy-corticosteroids as an index for these conditions. Marks and Leftin (5), however, were able to find butanol-extractable substances in human urine which gave rise to a positive Porter-Silber reaction without fulfilling the 17,21-dihydroxy-20-keto qualification. These findings raise an essential point of methodology: How much of the Porter-Silber chromogen in plasma or urine estimated by the simpler techniques may be ascribed to nonsteroidal material? Even with the Nelson and Samuels method as originally described, the Porter-Silber chromogen in blood plasma from normal subjects seldom exhibits a peak absorbancy at 410 mμ, whereas crystalline cortisol, even when extracted from water with chloroform and subjected to chromatographic treatment on Florisil columns, exhibits such a peak.

This paper will deal with the question of the interference of different substances frequently found in blood plasma on the formation of Porter-Silber chromogens and will describe a technique for the purification of blood plasma extracts employing a water-benzene partition.

MATERIALS AND METHODS

Materials

Chloroform U.S.P. (Merck) doubly distilled from K_2CO_3 immediately before use. Absolute ethanol U.S.P.

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Benzene (P.A.) distilled and thiophene-free.
Methylene chloride, Lot No. 390032 (Matheson, Coleman and Bell).
Sulfuric acid A.R. (Merck) diluted with distilled water (310:190).
Florisil (Floridin Company, Warren, Pennsylvania).
Phenylhydrazine-hydrochloride, recrystallized after purification with charcoal from 96 per cent ethanol. Twice recrystallized thereafter, dried over calcium chloride and kept in a desiccator.
Cortisol (Upjohn).
Methanol (Wasco) triple distilled from 2,4-diphenylhydrazine.
Cortisol-4-C^{14} (Tracerlab)[1]
Lactic acid, Lot No. 2672 (Mallinckrodt).
Ascorbic acid, Lot No. 00846 (Merck).
Anhydrous sodium sulfate, Lot No. 8024 (Mallinckrodt).
Human or dog plasma. The plasma was separated from the red cells immediately following withdrawal, using heparin as an anticoagulant, and then stored in a refrigerator for a maximum of fifteen days at a temperature not exceeding +2° C.

Methods

The extraction of blood plasma with an organic solvent, the preparation of Florisil, and the actual chromatographic separation have been described in detail in previous publications from our laboratory (2, 6). When using the Nelson-Samuels method, the Porter-Silber reaction was performed as in the publication of Nelson and Samuels, with the following changes: As observed in the recent work by Silber and Porter (7) redistilled methanol may, on occasion, cause a high background reading, whereas absolute ethanol will not. The latter was, therefore, used to dissolve the samples. After the ethanol and the phenylhydrazine-sulfuric acid were added to the samples, the results were more constant if a *covered* water bath was used for the subsequent incubation. The samples were then cooled in a covered waterbath at +10° C. for ten minutes and read at each of the following wavelengths: 370, 390, 410, 430 and 450 mμ, using a chloroform blank throughout the entire procedure to set the Beckman spectrophotometer to zero reading at each of the different wavelengths. Three different amounts (1 μg., 2 μg. and 3 μg.) of crystalline cortisol were chromatographed on standard Florisil columns in order to estimate the loss of material due to this procedure (2, 5, 8).

RESULTS

When the original Nelson-Samuels procedure was used, large amounts of glucose, lactose, ascorbic acid, vitamins A, D and E, and various salts added to plasma did not give rise to any color with the reagent. Only commercial lactic acid yielded a fraction extractable with chloroform and eluted from Florisil columns which produced a typical Porter and Silber reaction. Different techniques were employed to remove this fraction from the chloroform extract following addition of commercial lactic acid to plasma, but the only one which was successful involved a distribution between benzene and water. Talbot *et al.* (9) observed that in a partition between water and benzene, the distribution of crystalline cortisol and corti-

[1] Made available through the United States Public Health Service.

sone favored concentration of these substances in the water phase. This work, however, was carried out on relatively large amounts of steroids. Since cortisol is the predominant 17,21-dihydroxy-20-ketosteroid in the peripheral circulation of man (10), the following procedure was tried:

A 10-ml. aliquot of pooled plasma, with and without the addition of commercial lactic acid was extracted as described by Nelson and Samuels (2), chromatographed on Florisil columns and subjected to the Porter-Silber procedure. Some of these samples were treated as follows: Three extractions with 15 ml. of chloroform were performed on each sample. Each chloroform portion was transferred directly into a 50-ml. conical glass-stoppered centrifuge tube and evaporated to approximately 5 ml. in a waterbath not exceeding +50° C. The combined extract was taken to dryness after the third extraction. The residue was dissolved in 3 ml. of benzene, and 6 ml. of deionized water added. The tube was stoppered, shaken gently 100 times, and centrifuged at 450 rpm for five minutes. The water layer was then transferred to a 100-ml. centrifuge tube. All equipment coming in contact with the water-benzene mixture was carefully rinsed with water from a 30-ml. syringe, and the rinse water poured back into the conical centrifuge tube. This partition was repeated three times, using the same amount of water each time, and the water layers were collected and combined. The combined water layer from the partition was then extracted with chloroform three times, each extract being filtered into a 125-ml. Erlenmeyer flask, using Whatman filter paper No. 2 with some (approximately 2–3 Gm.) anhydrous sodium sulfate in the bottom of the filter. The chloroform was taken to dryness, the residue subjected to chromatography on Florisil columns and the Porter-Silber procedure carried out on the dry residue of the eluate containing 25 per cent methanol in chloroform (2). Table 1 shows that 100 mg. of commercial lactic acid added to 10 ml. of blood plasma could still produce significant color in the original Nelson-Samuels procedure, whereas a partition between water and benzene removed any interference due to this material.

To investigate the validity of the water-benzene partition, various tests were performed. The benzene phase from 100 ml. of human plasma was dried down following the partition with water, and subjected to paper chromatography (11). No cortisol or cortisone could be detected, using the ultraviolet scanning technique (12) or the triphenyltetrazolium test (13). Three samples (1, 2 and 3 μg.) of cortisol and an amount of cortisol-4-C^{14} equivalent to 200 counts per minute (c.p.m.) were each dissolved in triplicate samples of 10 ml. of human plasma which previously had been extracted with chloroform. The chloroform extracts of these samples were subjected to water-benzene partition. Cortisol-4-C^{14} was counted in the chloroform extract of the water layer from the partition, and the 17-hydroxycorticosteroids in the samples containing unlabeled material were determined by the Porter-Silber reaction after chromatography on Florisil. Table 2 substantiates the observation of Talbot *et al.* (9), inasmuch as the added material was recovered from the water phase of the partition within the limits of the methods used. The water and benzene phases from the partition of a chloroform extract of human plasma were taken to dryness

TABLE 1. DEGREE OF INTERFERENCE IN THE PORTER-SILBER REACTION INDUCED BY THE ADDITION OF 100 MG. OF COMMERCIAL LACTIC ACID TO 10-ML. ALIQUOTS OF POOLED HUMAN PLASMA

No.	Condition	Optical density at wavelengths of					
		370 mμ	390 mμ	410 mμ	430 mμ	450 mμ	Δ*
1	No lactic acid, N & S†	.100	.112	.108	.104	.072	.044
2	No lactic acid, N & S†	.062	.080	.080	.072	.052	.046
3	No lactic acid, N & S†	.046	.058	.054	.042	.020	.042
4	Lactic acid added	.120	.144	.146	.128	.095	.887
5	Lactic acid added	.135	.150	.153	.134	.105	.066
6	Lactic acid added	.204	.213	.216	.190	.148	.080
7	No lactic acid. Partition‡	.028	.050	.057	.048	.026	.050
8	No lactic acid. Partition‡	.032	.052	.060	.054	.036	.052
9	No lactic acid. Partition‡	.040	.056	.070	.060	.050	.050
10	Lactic acid added. Partition	.032	.049	.056	.047	.025	.055
11	Lactic acid added. Partition	.050	.060	.068	.058	.032	.054
12	Lactic acid added. Partition	.060	.075	.080	.070	.044	.056

*Δ = Twice the reading at 410 mμ divided by the sum of the readings at 370 mμ and 450 mμ.

† Determination by the original method of Nelson and Samuels.

‡ Partition between benzene and water was performed on the chloroform extract of this sample before chromatography on Florisil column.

and subjected to acetylation with acetic anhydride-C^{14} (14). Chromatography on paper was then performed on these fractions. The benzene fraction did not contain any acetylated steroids resembling the acetates of cortisol or cortisone.

The reproducibility of the method of Nelson and Samuels when a water-benzene partition is used prior to chromatography on Florisil, is shown in Table 3. There was little variation in the amount of Porter-Silber

TABLE 2. RECOVERY OF KNOWN AMOUNTS OF CRYSTALLINE CORTISOL OR CORTISOL-4-C^{14} IN THE WATER PHASE OF A WATER-BENZENE PARTITION ON CHLOROFORM EXTRACT OF HUMAN PLASMA

Substance	Amount added	Recovery %
Cortisol	1 μg.	86, 84, 83
Cortisol	2 μg.	91, 86, 89
Cortisol	3 μg.	90, 92, 89
Cortisol-4-C^{14}	200 c.p.m. above background	92, 90, 93

TABLE 3. CURVES OF PORTER-SILBER CHROMOGEN IN 10-ML. ALIQUOTS OF POOLED HUMAN PLASMA. ALL DETERMINATIONS WERE MADE BY THE NELSON-SAMUELS TECHNIQUE USING A WATER-BENZENE PARTITION.

Date	Technician	Optical density at the wavelengths of					
		370 mμ	390 mμ	410 mμ	430 mμ	450 mμ	Δ*
Feb. 3	R.H.	.270	.315	.319	.264	.168	.200
	R.H.	.248	.295	.302	.264	.155	.203
	R.H.	.230	.271	.279	.230	.130	.198
Feb. 5	K.B-J.	.149	.196	.225	.180	.111	.190
	K.B-J.	.170	.219	.240	.200	.120	.190
	K.B-J.	.140	.199	.224	.180	.110	.198
Feb. 7	J.v.D.	.200	.250	.260	.210	.125	.195
	J.v.D.	.230	.277	.290	.235	.140	.205
	J.v.D.	.231	.279	.285	.231	.140	.199

* See footnotes to Table 1.

chromogen recovered from pooled blood plasma by three different workers on three separate occasions using various batches of Florisil. Notable are the distinct peaks at 410 mμ and the rather narrow range among the Porter-Silber chromogen curves.

In 1954 Silber and Porter described a method for the determination of plasma and urinary 17-hydroxycorticosteroids not employing chromatographic separation (7). Peterson and Wyngaarden (15) substituted purified methylene chloride for chloroform in the method of Silber and Porter. This change of organic solvent has improved the method of Silber and Porter considerably, and low blank readings and reproducible results can easily be obtained. In plasma from the normal human subject, this method offers distinct advantages over the method of Nelson and Samuels, since it is less time-consuming and avoids the loss of 17,21-dihydroxy-20-ketosteroids on Florisil columns. We have found, however, that in plasma obtained from subjects suffering from diseases of the liver and those subjected to various forms of severe change in body environment—"stress"—there are pigments or unknown substances which cannot be removed by the purification steps described by Silber and Porter and which will interfere in the final development of the Porter-Silber chromogen in such plasma. Other workers have stated that certain drugs will also interfere. We have therefore introduced the water-benzene partition into this method; this seems to achieve a purified Porter-Silber chromogen without the use of column chromatography.

A 4-ml. sample of plasma is diluted to 10 ml. with deionized water. If high levels of 17-hydroxycorticosteroids (above 20 μg. per 100 ml. of plasma) are to be expected (16–20), 1 ml. of plasma diluted to 10 ml. with water may be used advantageously. The plasma is extracted three times with 15 ml. of methylene chloride (substituted for chloroform), as described by Nelson and Samuels (2), and the combined methylene-chloride layer is dried down to approximately 30–40 ml. in a conical glass-stoppered centrifuge tube. After the addition of 2.0 ml. of 0.1 *N* NaOH to the methylene chloride, the sample is shaken for two minutes and centrifuged. The methylene-chloride layer is then transferred to a clean conical centrifuge tube and taken to dryness. The water-benzene partition previously described is performed on the dry methylene-chloride residue, and the water phase from the partition is extracted three times with 10-ml. portions of methylene chloride. The combined methylene chloride layers are transferred to a conical centrifuge tube through a small filter containing anhydrous sodium sulfate. To each tube is added 0.3 ml. of a solution comprising 10 mg. of recrystallized phenylhydrazine dissolved in absolute ethanol-73.6% sulfuric acid (1:2). After the addition of the Porter-Silber reagent, the tubes are shaken vigorously for two minutes, and centrifuged for ten minutes. The methylene-chloride layer on top is removed by suction, using a vacuum pump with a trap. The samples are then incubated and read at 370, 390, 410, 430 and 450 mμ. As a blank, a sample of 10 ml. of deionized water is taken through the entire procedure. A reference standard of 0.5 μg. of crystalline cortisol dissolved in 10 ml. of deionized water is also carried through the procedure. Table 4 indicates that closely reproducible results can be obtained on pooled blood plasma from subjects suffering from liver diseases and other abnormal conditions. No major difference in values for plasma levels of 17-hydroxycorticosteroids is found between the method of Nelson and

TABLE 4. CURVES OF PORTER-SILBER CHROMOGEN IN POOLED HUMAN PLASMA. ALL DETERMINATIONS WERE MADE IN TRIPLICATE, BY THE MODIFIED METHOD OF SILBER AND PORTER USING A BENZENE-WATER PARTITION

Condition	Volume extracted (ml.)	Optical density at the wavelengths of					
		370 mμ	390 mμ	410 mμ	430 mμ	450 mμ	Δ*
Normal	5	.116	.140	.154	.137	.098	.094
	5	.086	.113	.127	.107	.073	.095
	5	.083	.114	.129	.111	.075	.100
Chlorpromazine-treated	5	.050	.070	.081	.074	.045	.067
	5	.058	.079	.094	.083	.059	.071
	5	.050	.070	.080	.073	.045	.065
Cirrhosis hepatitis	5	.056	.069	.083	.069	.040	.070
	5	.054	.066	.077	.064	.035	.065
	5	.040	.060	.071	.060	.043	.069
3 days post partum	1	.080	.087	.094	.070	.034	.074
	1	.094	.113	.111	.090	.048	.080
	1	.090	.100	.105	.084	.040	.080

* See footnotes to Table 1.

TABLE 5. PLASMA LEVELS OF 17-HYDROXYCORTICOSTEROIDS* DETERMINED BY THE NELSON-SAMUELS METHOD AND BY THE MODIFIED METHOD OF SILBER AND PORTER. A WATER-BENZENE PARTITION IS USED IN BOTH PROCEDURES.

Condition	17-OHCS (μg. per 100 ml. of plasma)	
	N & S method	Mod. S & P method
Normal	10.6	9.4
Normal	8.9	10.1
Normal	12.4	11.1
3 days post partum	22.1	21.4
3 days post partum	34.6	32.3
3 days post partum	39.0	37.6
Female dog control	2.3	1.8
6 days hypophysect'd	0.3	0.0

* The levels of 17-hydroxycorticosteroids were calculated on the basis of the recovery of known amounts of cortisol carried through both methods.

Samuels and the method of Silber and Porter as modified by Peterson and Wyngaarden, if the benzene-water partition is used in both methods (Table 5). Crystalline cortisol in amounts varying from 0.1 to 1.6 μg. has been recovered in the range from 94 to 100 per cent by the modified Peterson and Wyngaarden method, and Table 6 indicates the high degree of reproducibility.

DISCUSSION

As Silber and Busch (21) have reported, most constituents which could conceivably be in plasma do not interfere with the Porter-Silber reaction. Yet interference has been reported (5). In the present studies, addition of

TABLE 6. PORTER-SILBER CHROMOGEN CURVES FROM DIFFERENT AMOUNTS OF POOLED HUMAN PLASMA. ALL SAMPLES SUBJECTED TO THE MODIFIED SILBER-PORTER METHOD WITH A WATER-BENZENE PARTITION.

Volume extracted (ml.)	Optical density at the wavelengths of						
	370 mμ	390 mμ	410 mμ	430 mμ	450 mμ	Δ*	Δ/ml.
2	.058	.063	.066	.057	.038	.036	.018
4	.086	.103	.108	.093	.063	.067	.017
6	.125	.146	.159	.135	.088	.105	.018
8	.140	.175	.190	.060	.105	.135	.017
10	.168	.210	.233	.198	.127	.171	.017

* See footnotes to Table 1.

ordinary reagent lactic acid yielded increased chromogen, using the method of Nelson and Samuels. This interference can be overcome, however, by the partition and subsequent chromatography on Florisil.

The method of Nelson and Samuels as described in this paper has been used as a standard procedure for the determination of plasma 17-hydroxycorticosteroids for fifteen months in our laboratory. Based on results with approximately 300 individual control plasma samples from the normal human subject, it is felt that the Porter-Silber absorbance curves are less distorted following the introduction of the water-benzene partition step. Even at minimal levels of 17-hydroxycorticosteroids in the plasma, a peak absorption at 410 mμ is seen. Since cortisol exhibits a peak at this wavelength in the Porter-Silber reaction, *all unknown samples should be read at five different wavelengths*, two of these readings being at either side of the peak absorbancy. This makes possible the recognition of excessive background color. To depend upon one wavelength alone for the estimation of plasma 17-hydroxycorticosteroids, is dangerous and may easily lead to erroneous results. All unknown samples exhibiting greater absorbancy at 390 mμ than at 410 mμ should be looked upon with skepticism.

Previously, our group reported normal plasma levels of 17,21-dihydroxy-20-ketosteroids in man, ranging from 5 μg. to 23 μg. per 100 ml. (22). With improvement in method and better control of subjects over the last four-year period this normal span for adults between 20 and 50 years of age has decreased considerably. At present, when samples are drawn between 9 A.M. and 2 P.M., the range seems to be from 7 μg. to 15 μg. per 100 ml. of plasma. In the fasting subject, any level of these steroids above 18 or 20 μg. per 100 ml. should be considered pathologic unless proved otherwise.

In comparing the method of Nelson and Samuels employing column chromatography with the modified method of Silber and Porter (7, 15), the latter offers certain distinct advantages if the water-benzene purification is used. It is less expensive and less time-consuming, making possible a greater number of analyses during one day. Further, this procedure can be performed with less loss of 17,21-dihydroxy-20-ketosteroids than in the original method of Nelson and Samuels, and a smaller amount of plasma is needed. For plasma levels of these steroids, both methods will yield values which are within the same range. These techniques are therefore reliable for the measurement of adrenocortical capacity and catabolism of steroids having a 17,21-dihydroxy-20-keto configuration. The method of Nelson and Samuels may still, however, be the more valuable method in certain studies, since fractions containing other steroids (such as 17-ketosteroids) can be eluted separately from those producing the Porter-Silber reaction (23). Also, if excess amounts of chromogens are present—

as occasionally found after the plasma has been incubated with β-glucuronidase—a purification over Florisil may be of value.

We should like to re-emphasize one point (7): The methods herein described remain micromethods and require the skill and care characteristic of all such methods. A scrupulous washing of glassware is necessary, including a soaking procedure in chromic acid for twenty-four hours followed by a thorough rinsing with tap water (20 times) and with distilled water (10 times), and drying in air. Technical training in the handling of pooled plasma and in the recovery of crystalline cortisol is also of importance if reliable results are to be obtained. In our laboratory, technicians are trained for three to four months in these procedures, regardless of previous training or laboratory background, before it is felt that they are in a position to be proficient in determining plasma 17-hydroxycorticosteroid levels using the Porter-Silber reaction.

SUMMARY

A water-benzene partition of the organic-solvent extract of blood plasma has been introduced as a step in purification for the determination of plasma 17-hydroxycorticosteroid concentration using the Porter-Silber color reaction for 17,21-dihydroxy-20-ketosteroids. This was undertaken in order to eliminate the presence of interfering background material and other substances in plasma which are capable of yielding a positive Porter-Silber chromogen. Employing this partition, the method of Nelson and Samuels with chromatography on Florisil was compared to the modified method of Silber and Porter without chromatographic separation. For routine determinations of the level of plasma 17-hydroxycorticosteroids, the latter method is to be preferred. In certain studies, however, chromatography on Florisil will offer advantages.

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REFERENCES

1. Porter, C. C., and Silber, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201, 1950.
2. Nelson, D. H., and Samuels, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12**: 519, 1952.
3. Glenn, E. M., and Nelson, D. H.: Chemical method for the determination of

17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13**: 911, 1953.

4. Reddy, W. J.; Jenkins, D., and Thorn, G. W.: Estimation of 17-hydroxycorticoids in urine, *Metabolism* **1**: 511, 1952.
5. Marks, L. J., and Leftin, J. H.: A note of caution on the lack of specificity of the Porter-Silber reaction for 17,21-dihydroxy-20-ketosteroids, *J. Clin. Endocrinol. & Metab.* **14**: 1263, 1954.
6. Eik-Nes, K.; Nelson, D. H., and Samuels, L. T.: Determination of 17,21-dihydroxycorticosteroids in plasma, *J. Clin. Endocrinol. & Metab.* **13**: 1280, 1953.
7. Silber, R. H., and Porter, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923, 1954.
8. Leftin, J. H.; Marks, L. J., and Leonard, M. P.: The question of the destruction of 17,21-dihydroxycorticosteroids by phosgene, *J. Clin. Endocrinol. & Metab.* **15**: 377, 1955.
9. Talbot, N. B.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. K.: The colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160**: 535, 1945.
10. Bush, I. E., and Sandberg, A. A.: Adrenocortical hormones in human plasma, *J. Biol. Chem.* **205**: 783, 1953.
11. Bush, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissue, *Biochem. J.* **50**: 370, 1952.
12. Haines, W. J., and Drake, N. A.: Fluorescence scanner for evaluation of paper chromatograms of adrenal cortical hormones, *Fed. Proc.* **9**: 180, 1950.
13. Zaffaroni, A.: Micromethods for the analysis of adrenocortical steroids, *Recent Prog. Hormone Res.* **8**: 51, 1953.
14. Berliner, D. L.: Micromethod for the determination of acetylable steroids from blood and urine, *Fed. Proc.* **15**: 219, 1956.
15. Peterson, R. E., and Wyngaarden, J. B.: Personal communication, 1955.
16. Eik-Nes, K.; Sandberg, A. A.; Nelson, D. H.; Tyler, F. H., and Samuels, L. T.: Changes in plasma levels of 17-hydroxycorticosteroids during the intravenous administration of ACTH. I. A test of adrenocortical capacity in the human, *J. Clin. Invest.* **33**: 1502, 1954.
17. Brown, H.; Willardson, D. G.; Samuels, L. T., and Tyler, F. H.: Corticosteroid metabolism in liver disease, *J. Clin. Invest.* **33**: 1524, 1954.
18. Bayliss, R. I.; Browne, J. C.; Round, B. P., and Steinbeck, A. W.: Plasma 17-hydroxycorticosteroids in pregnancy, *Lancet* **1**: 62, 1955.
19. Klein, R.; Papadatos, C., and Fortunato, J.: Serum corticoids in children under stress, *J. Pediat.* **43**: 385, 1953.
20. Franksson, C., and Gemzell, C. A.: Blood levels of 17-hydroxycorticosteroids in surgery and allied conditions, *Acta chir. scandinav.* **106**: 24, 1953.
21. Silber, R. H., and Busch, R. D.: The specificity of the reaction of phenylhydrazine with 17,21-dihydroxy-20-ketosteroids, *J. Clin. Endocrinol. & Metab.* **15**: 505, 1955.
22. Bliss, E. L.; Sandberg, A. A.; Nelson, D. H., and Eik-Nes, K.: The normal levels of 17-hydroxycorticosteroids in the peripheral blood of man, *J. Clin. Invest.* **32**: 818, 1953.
23. Migeon, C. J., and Plager, J. E.: A method for the fractionation and measurement of 17-ketosteroids in human plasma, *J. Clin. Endocrinol. & Metab.* **15**: 702, 1955.

DETERMINATION OF CORTICOTROPIN (ACTH) IN HUMAN BLOOD AND URINE BY A MODIFIED OXYCELLULOSE METHOD

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THE method of Sydnor and Sayers (1) for the determination of corticotropin (ACTH) in blood has been modified and shortened so that the entire procedure of concentrating corticotropin in an injectable volume can be completed within ten hours. With this new modification we have been able to inject the equivalent of 200 ml. of blood into a single test rat. The largest volume of blood which could be used by Sydnor and Sayers was 40 ml.; and in normal subjects, corticotropin was undetectable in this amount (2, 3). By decreasing the amount of acetic acid mixed with blood and centrifuging the mixture before adding oxycellulose, we have succeeded in removing toxic material which precluded the injection of volumes larger than 40 ml. equivalent. The modification is based on the technic developed by Takahashi for the determination of MSH in blood (4).

The oxycellulose technic has also been applied to urine, so that the equivalent of a 24-hour urine sample can be tested in a single rat.

METHOD

Blood

To 200 ml. of freshly drawn blood was added 60 ml. of glacial acetic acid. The mixture was stirred, diluted to 900 ml. by adding distilled water, and maintained with constant stirring at 70° C. for thirty minutes. It was then centrifuged at 3500 r.p.m. for fifteen minutes. The supernatant liquid was decanted and the pH was adjusted to 3.3 by the addition of glacial acetic acid or water. The precipitate was discarded. One gram of oxycellulose[1] was added to the supernatant fraction. The mixture was stirred for six

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[1] COOH content, 10 to 12 per cent. Supplied by Eastman Chemical Products, Kingsport, Tennessee. Washed before use with 0.1 N acetic acid, water, 0.1 N HCl, water and 0.1 N acetic acid.

hours, and then filtered on a coarse sintered glass funnel. The oxycellulose on the funnel was washed with 0.1 *N* acetic acid until the washings were colorless.

Elution was carried out either by column or by batch method. In the column method, oxycellulose was packed in a column of 7 mm. diameter and washed with 5 ml. of distilled water. A 2-ml. portion of 0.1 *N* HCl was then passed at a rate of approximately 1 ml. per fifteen minutes, followed by 5 ml. of distilled water at the same rate. The effluent from the column was tested with "universal pH paper" and all fractions with pH 2 or less were collected. In the batch method, corticotropin was eluted by stirring oxycellulose with 3 ml. of 0.1 *N* HCl at intervals for an hour. The mixture was then filtered through a medium sintered glass funnel. Further elution was then carried out with another 2 ml. of 0.1 *N* HCl.

Urine

A 24-hour specimen of urine collected over 60 ml. of glacial acetic acid was filtered, and the pH adjusted to 3.3. One gram of oxycellulose was then added. After stirring six hours, the process of elution described for blood was followed.

All samples were assayed by the ascorbic-acid depletion method (5).

RESULTS

Recovery Experiments

Transfusion blood

A 2-milliunit sample of corticotropin (ACTH) was added to 200 ml. of outdated transfusion blood and the procedure of concentrating corticotropin was carried out as described. R represents the ratio of potency of the recovered sample compared to standard ACTH, and the range in parenthesis shows the limit of one standard error:

Date & Method	Ascorbic-acid depletion (mg./100 Gm.)	λ	b	s	R
I. *Feb. 15, 1956. Column method*					
Recovered sample		0.23	145	33	
2.0 mill. u.	139, 135, 100, 99				
					0.97
0.5 m.u.	65, 25, 93, 43, 55				(1.26–0.75)
Standard ACTH		0.19	100	19	
2.0 m.u.	143, 142, 102				
0.5 m.u.	80, 76, 47, 72				
Blood blank	−35, 10, −5				

Date & Method	Ascorbic-acid depletion (mg./100 Gm.)	λ	b	s	R
II. *Feb. 22, 1956. Column method*					
Recovered sample		0.39	145	56	
2.0 m.u.	122, 156, 157, 190, 30				
					1.05
0.5 m.u.	21, 102, −5, 55				(1.54–0.73)
Standard ACTH		0.23	130	30	
2.0 m.u.	172, 132, 106, 100				
0.5 m.u.	51, 57, 79, 13				
III. *Feb. 7, 1956. Column method (only 0.5 Gm. oxycell. used; eluted with 1. ml. 0.1 N HCl)*					
Recovered sample		0.35	273	96	
2.0 m.u.	199, 297, 114, 35				
					0.62
0.5 m.u.	−59, 67, −19				(0.90–0.43)
Standard ACTH		0.24	156	39	
2.0 m.u.	147, 162, 180, 129, 251				
0.5 m.u.	49, 124, 46, 128, 57, 50				
IV. *Mar. 6, 1956. Column method (stirring cont'd only 2 hrs.)*					
Recovered sample		0.39	111	43	
2.0 m.u.	121, 158, 91, 100				
					1.03
0.5 m.u.	25, 124, 3				(1.39–0.77)
Standard ACTH		0.06	175	11	
2.0 m.u.	145, 126, 135, 122				
0.5 m.u.	39, 29, 10				
V. *Mar. 20, 1956. Column method (stirring cont'd only 2 hrs.)*					
Recovered sample		0.22	130	28	
2.0 m.u.	128, 60, 130, 107, 104				
					0.70
0.5 m.u.	67, −12, 26, 29				(1.07–0.46)
Standard ACTH		0.42	155	65	
2.0 m.u.	82, 73, 229, 152				
0.5 m.u.	−9, 140, 26, −3				
Blood blank	4, −16, 55				
VI. *Apr. 17, 1956. Column method*					
Recovered sample		0.20	152	30	
2.0 m.u.	200, 153, 174, 114, 207				
					0.89
0.5 m.u.	70, 102, 78, 65				(1.20–0.67)
Standard ACTH		0.27	154	42	
2.0 m.u.	254, 187, 134, 196				
0.5 m.u.	41, 58, 95				
Blood blank	−36, 11, 3				

Blood from normal subjects

Name	Age & Sex	Ascorbic-acid depletion by 200 ml. (mg./100 Gm.)	Ascorbic-acid depletion by standard ACTH (0.25 m.u.)*
T.C.	35 M	−36	73, −6, 18, 31, 7, 106 (Av., 38): 0.5 m.u.
J.C.	28 M	96	
P.L.	30 M	22	
T.F.	26 M	1)† 55	
		2) 30	35, 102, 7, 92, 46, 27 (Av., 51)
M.D.	72 F	26	
W.P.	25 M	109, 119	58, 42, 85, 85, 15, 41 (Av., 65)
B.T.	22 F	1) −40	
		2) 32, −1	35, 102, 7, 92, 46, 27 (Av., 51)
D.W.	22 M	1) 64	58, 42, 85, 85, 15, 41 (Av., 65)
		2) −46	35, 102, 7, 92, 46, 27 (Av., 51)
V.P.	24 F	84, 64	
S.G.	18 M	32	
M.G.	34 F	70, 37	58, −3, −3, 80, 37, 22 (Av., 32)
K.D.	24 M	38	
W.D.	27 M	1) −5	
		2) 115	35, 102, 7, 92, 46, 27 (Av., 51)
W.G.	34 M	1) 16, 28	58, −3, −3, 80, 37, 22 (Av., 32)
		2) 48, 58	35, 102, 7, 92, 46, 27 (Av., 51)
W.C.	30 M	149	
E.H.	28 F	21	3, 101, 104, 48, 42 (Av., 59)
M.F.	24 F	51	
J.F.‡	21 F	1) 145	58, 45, 85, 85, 15, 41 (Av., 65)
		2) 11, 6	3, 101, 104, 48, 42 (Av., 59)
M.B.	26 F	82, 16	
L.K.	29 F	66, 5	
N.E.	26 F	−35, −42	
S.L.	26 M	−12, 50	35, 102, 7, 92, 46, 27 (Av., 51)
B.K.	19 F	85, 62	
M.Z.	39 F	7, 9	
R.D.	30 F	55, 56	
J.T.	27 M	72, 52 (133 ml.)	23, 31, 79, 45 (Av., 45)
M.K.	59 F	70, 75, 144 (100 ml.)	

* Ascorbic-acid depletion in response to physiologic sodium chloride was as follows: −36, −1, −39,14,26, −44, −3, −19, −17,8,16,54, −30,19,10, −17, −2,10,16,24,5,9,46.

† Samples 1 and 2 were taken on different occasions.

‡ Subject J.F. had a mild upper respiratory infection when sample 1 was drawn but was completely recovered when sample 2 was taken.

Urine (normal subjects unless otherwise specified)

Name	Age & Sex	Collection period (hrs.)	Ascorbic-acid depletion (mg./100 Gm.)	Ascorbic-acid depletion by standard ACTH (0.25 m.u.)
T.F.	26 M	24	45, 22, 47, 48, 35, 79, 87, 53, 30, 50	6, 91, 92, 59 (Av., 62)
J.B.	27 M	24	186, 121	3, 101, 104, 48, 42 (Av., 59)
C.H.	53 M	24	61	113, 100, 86, 86 (Av., 96)
S.L.	26 M	24	84	
F.S.	51 M	24	26	
G.Z.	44 F	24	83	
		12	72, 10	
R.B.	53 M	12	−2, −8	
H.B.	46 M	12	12	
V.K.	28 M	12	−41, 12	
F.S.*	45 F	12	69, 59	
B.E.†	20 F	12	68	
M.K.†	56 F	12	27	

* Hypertension.
† Thyrotoxicosis.

DISCUSSION

Earlier observations on the determination of corticotropin in blood (6–11), using small amounts of blood or plasma either directly or after extraction by the acid-acetone technic (12), indicated a high concentration (30–200 milliunits per 100 milliliters of blood). Recently, lower levels of corticotropin by the same method were also reported. Parrott (13) was unable to find corticotropin in 4 ml. of plasma by a modification of the acid-acetone technic, an observation which may be interpreted as demonstrating less than 2.5 m.u. per 100 ml. of blood. Gaarenstroom *et al.* (14) used the equivalent of 20 ml. of plasma prepared by the same method, and ascorbic-acid depletions of more than 60 mg. per 100 Gm. were observed in 7 of 33 normal subjects; this might indicate less than 0.5 m.u. of corticotropin per 100 ml. of blood. After an extensive review of literature, as well as their own studies using direct injection of plasma, Paris and associates (15) concluded that corticotropin was not demonstrable in normal blood.

The oxycellulose technic, although yielding adequate and consistent recoveries, results in lower values than with other methods. Corticotropin has not been detectable in 40 ml. of blood obtained from normal or febrile patients (2). Sayers (16) concluded that this low concentration was consistent with the results of clinical studies with intravenous administration of corticotropin, in which less than 12 m.u. of corticotropin per 100 ml. of blood seemed to induce a maximum adrenal response.

In our assays, the ascorbic-acid depletion induced by 0.25 m.u. of corticotropin was significantly different from that induced by physiologic sodium chloride ($t=7.1$). Taking all samples of blood as a group (52 samples; average, 43 mg. of ascorbic-acid depletion per 100 Gm. of tissue, with a standard deviation of 48 and standard error of 6.7), and comparing this with a group of blood samples from subjects receiving injections of physiologic saline (23 samples; average, 2 mg. of ascorbic-acid depletion, with a standard deviation of 25 and a standard error of 5.3), a t value of 4.8 was obtained. The difference between the means is highly significant (P less than 0.01). Comparing blood samples with 0.25 m.u. of standard corticotropin (36 samples; average, 53 mg. of ascorbic-acid depletion, with a standard deviation of 35 and a standard error of 5.8), the t value was 1.1—no significant difference. It appears, therefore, that the average concentration of corticotropin in normal human blood is approximately 1.0 milliunit per liter.

Individual differences appear to be great. In Subject W. P. there was a detectable amount of corticotropin in 200 ml. of blood. In Subjects W. C., M. K., V. P. and B. K. the results also suggested the presence of corticotropin in the blood. In the other subjects, the evidence was less definite.

The presence of corticotropin in urine has been a matter of even more controversy. Locke, Albert and Kepler (17) did not consistently find corticotropin in normal urine. Rubin, Dorfman and Dorfman (18) reported a very high value for corticotropin (more than 1 unit per liter) in fresh urine, using the Munson modification of the ascorbic-acid depletion method. This observation has not been confirmed by others.

Our study indicates a significant difference between the results with physiologic saline and with 24-hour urine (16 samples; average, 66 mg. of ascorbic-acid depletion, with a standard deviation of 42 and a standard error of 10.4)—the t value being 5.5. Against 0.25 m.u. of corticotropin, the t value was 1.1 and the difference was insignificant. The amount of corticotropin appearing in the urine of normal subjects appears to be of the order of 0.25 milliunit per day.

SUMMARY

A simplification of the oxycellulose technic for the determination of corticotropin (ACTH) in blood is described.

In 5 of 27 normal subjects there were detectable amounts of corticotropin in 200-ml. samples of blood. The average value for the concentration of corticotropin in the blood of normal subjects appears to be 1 milliunit per liter.

The oxycellulose technic was also applied to urine. Approximately 0.25 milliunit of corticotropin was found per 24-hour specimen in normal subjects.

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REFERENCES

1. Sydnor, K. L., and Sayers, G.: A technic for determination of adrenocorticotrophin in blood, *Proc. Soc. Exper. Biol. & Med.* **79**: 432, 1952.
2. Sydnor, K. L.; Sayers, G.; Brown, H., and Tyler, F. H.: Preliminary studies on blood ACTH in man, *J. Clin. Endocrinol. & Metab.* **13**: 891, 1953
3. Sydnor, K. L.; Kelley, V. C.; Raile, R. B.; Ely, R. S., and Sayers, G.: Blood adrenocorticotrophin in children with congenital adrenal hyperplasia, *Proc. Soc. Exper. Biol. & Med.* **82**: 695, 1953.
4. Takahashi, Y.: Personal communication.
5. Sayers, M. A.; Sayers, G., and Woodbury, L. A.: The assay of adrenocorticotrophic hormone by the adrenal ascorbic acid-depletion method, *Endocrinology* **42**: 379, 1948.
6. Bornstein, J., and Trewhella, P.: Adrenocorticotropic activity of blood plasma extracts, *Lancet* **2**: 678, 1950.
7. Montanari, L.; Martinelli, M.; Rossi, C. A., and Moruzzi, G.: Adrenocorticotropic activity of plasma, *J.A.M.A.* **147**: 525, 1951.
8. Parrott, D. M. V.: ACTH-like activity of plasma extracts, *J. Endocrinol.* (British) **7**: lxxx, 1951.
9. Gray, C. H., and Parrott, D. M. V.: Observations on a method of measuring adrenocorticotrophic hormone in plasma, *J. Endocrinol.* (British) **9**: 236, 1953.
10. Rossi, C. A.; Montanari, L.; Martinelli, M., and Moruzzi, G.: Ultrafiltrable ACTH activity of human plasma, *J.A.M.A.* **149**: 1242, 1952.
11. Ceresa, F., and Reyneri, C.: L'attivita corticotropa del plasma umano. Allestimento delle curne campione e ricerca dei valori fisiologici con il metado di Bornstein e Trewhella, *Folia endocrinol.* **5**: 179, 1952.
12. Lyons, W. R.: Preparation and assay of mammotropic hormone, *Proc. Soc. Exper. Biol. & Med.* **35**: 645, 1937.
13. Parrott, D. M. V.: The assay of adrenocorticotrophic activity in plasma extracts, *J. Endocrinol.* (British) **12**: 120, 1955.
14. Gaarenstroom, J. H.; Groen, A., and DeWied, D.: The relation between "ACTH content" of the blood and the urinary excretion of 17-ketosteroids, *Acta endocrinol.* **17**: 89, 1954.
15. Paris, J.; Upson, M., Jr.; Sprague, R. G.; Salassa, R. M., and Albert, A.: Corticotropic activity of human blood, *J. Clin. Endocrinol. & Metab.* **14**: 597, 1954.
16. Sayers, G.: Blood ACTH (Editorial), *J. Clin. Endocrinol. & Metab.* **15**: 754, 1955.
17. Locke, W.; Albert, A., and Kepler, E. J.: Adrenotrophic activity of human urine, *Proc. Soc. Exper. Biol. & Med.* **72**: 470, 1949.
18. Rubin, B. L.; Dorfman, R. I., and Dorfman, A.: Adrenocorticotropic hormone (ACTH) in urine, *J. Clin. Endocrinol. & Metab.* **14**: 154, 1954.

EXCRETION OF URINARY CORTICOIDS IN CONDITIONS OF CHRONIC ASTHENIA

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IT HAS been postulated that some disturbance in the secretion of the adrenal cortex is present in conditions of chronic asthenia. Several arguments seem to corroborate this theory; for example, asthenia is one of the major symptoms of adrenal insufficiency. Furthermore, recent researches on the role of the adrenals in fatigue and resistance to stress are in harmony with the concept of a general hypofunction of these glands in asthenia.

In fact the problem is quite complicated, since investigations on the adrenals with classical tests in fatigue conditions have not revealed gross alterations in the level of blood sugar and in electrolyte balance such as are observed in Addison's disease.

A new light has been shed on the functions of the adrenals by recent studies on corticosteroidogenesis (1, 2). It seems possible to determine whether the constant minor aggressions of everyday life and the major toxic, traumatic or infectious stresses may eventually disturb adrenocortical metabolism and thus unbalance the elaboration of corticoids. Defects of corticosteroidogenesis have been found in other pathologic states (3, 4, 5).

The present study provides some evidence for this hypothesis from the study of patients who had undergone a series of setbacks in their professional, social or emotional life.

MATERIAL AND METHODS

The study was carried out on 34 normal subjects and 52 subjects with chronic asthenia. None of the latter had any disease to which one might ascribe the asthenia, and any obvious endocrine disturbances (such as thyroid or adrenal insufficiency or diabetes), cancer, tuberculosis, cardiovascular disorders or any other major disease were carefully ruled out. Table 1 lists the diagnoses, based on the clinical history. These patients complained of general asthenia, involving somatic, sexual and psychic activity. Otherwise, they were healthy and able to carry on a gainful occupation. All of them showed minor psychologic disturbance in relation to psychic trauma or maladaptation to the social or emotional aspects of their life.

A. The function of the adrenal cortex was investigated by measuring the urinary

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corticoids under basal conditions and after administration of corticotropin; changes in total eosinophil count were noted, in parallel.

B. Urinary 17-ketosteroid excretion was measured by the Talbot-Jayle modification of the original Callow technic (6, 7).

C. In 40 cases corticoid output (C_{21}-α-ketol corticoids and 17-hydroxycorticoids) was determined by a method described by Romani (8), involving acid hydrolysis of urines for twenty-four hours at 37° C. and chloroform extraction; and in 12 cases by a modification of this method described by Romani *et al.* (9), involving hydrolysis of urine samples with beta-glucuronidase and sulfatase, chloroform extraction and chromatographic separation of the C_{21}-α-ketol corticoids on Florisil. In both procedures we estimated total reducing corticoids with the Folin-Wu reagent, and 17,21-dihydroxy-20-ketones with the phenylhydrazine-sulfuric reagent of Porter and Silber (10). In each

TABLE 1

Diagnosis	No. of cases
Industrial fatigue, including professional maladaption, disturbed interpersonnel relations, or overwork	30
Ex-prisoner of war	3
Constitutional psychasthenia	5
Chronic asthenia, simulating endocrine insufficiency	7
Chronic asthenia with slight neurologic symptoms	3
Asthenia with obesity	2
Subjects recovering from infections	2

method, urinary 17-desoxycorticoids (involving end-products of the metabolism of corticosterone and desoxycorticosterone) were estimated by calculating the difference between the total reducing corticoids (T.R.C.) on the basis of the Folin-Wu chromogens, and the 17,21-dihydroxy-20-ketones (17-hydroxy or Porter-Silber chromogens) in the same Florisil eluate (chloroform-ethanol 20 per cent). On the basis of paper chromatographic separation, enzymatic hydrolysis yields an excess of reducing material with the Folin-Wu reagent, which corresponds to the different reducing power of hydrocortisone metabolites; this excess has been estimated as 20 per cent of the value of the Porter-Silber chromogens. Therefore, after enzymatic hydrolysis, it is necessary to subtract this excess from the value for total Folin-Wu chromogens (corrected T.R.C.). Of 34 normal subjects, studied in association with the asthenics, 19 were investigated with the first technic (acid hydrolysis) and 15 with the second technic (enzymatic hydrolysis).

D. The adrenal cortex was stimulated by intramuscular injection of 40 units of a long-acting ACTH (corticotropin-zinc phosphate, Organon). Previous reports by other authors have shown that this preparation induces an increase in the level of plasma 17-hydroxycorticoids lasting at least twelve hours (11) and an eosinopenia culminating at the eighth hour (12). Total eosinophil counts were made on blood samples taken before, and at six and eight hours after injection of ACTH. Urinary excretions of 17-ketosteroids and of corticoids were determined in the 24-hour period immediately before and after this injection.

In order to ascertain the disturbance in the excretion of α-ketol corticoids (ratio of 17-hydroxy catabolites to 17-desoxy catabolites), in 6 selected cases, we made a more

precise study of corticoid excretion, using paper chromatographic separation of the corresponding fractions of the urinary extracts. Paper chromatography determinations were carried out with the Romani adaptation of the technic of Zaffaroni, involving: 1) enzymatic hydrolysis of the urine (about a third of a 24-hour specimen) and isolation with Florisil chromatography of the C_{21}-α-ketol fraction, 2) double paper chromatography on toluene/propylene-glycol, and 3) estimation, on eluates of each fraction identified on the paper strips, of the relative migration (RDOC), group reaction (reduction of blue tetrazolium and determination of Porter-Silber chromogens) and absorption spectra in concentrated sulfuric acid (13, 14, 15). In 3 normal subjects and 6 subjects suffering of chronic asthenia it was found that the relative proportion of F catabolites and B catabolites could be estimated with sufficient accuracy in this manner.

RESULTS

I. *Normal subjects*

17-Ketosteroids

In 30 normal subjects, urinary 17-ketosteroids were uniformly higher in males than in females, corroborating previous studies. For males, the mean value was 16 mg. in twenty-four hours, with a range of 5 mg. between the extremes; for females, the values were significantly lower, the mean being about 10 mg., with a range of 5.5 mg.

Corticoids

The results with acid hydrolysis were different from those with enzymatic hydrolysis (glucuronidase+sulfatase). After acid hydrolysis of the urines, the mean value (17-OH and T.R.C.) was slightly higher in males (4.7 mg.) than in females (3.7 mg.). There was remarkable concordance (within less than 0.5 mg.) in the values found for 17-OH and T.R.C. (16), suggesting that the major part of alpha-ketolic metabolites comes from compound F (tetrahydrocortisone and tetrahydrocortisol).

The effect of administration of corticotropin in normal subjects is characterized by a severe eosinopenia; the increase in urinary corticoids, using acid hydrolysis, does not furnish a complete estimation of adrenal activation, as the recovery of alpha-ketolic metabolites seems to be insufficient. Paper chromatography of acid urinary extracts shows degraded products of corticoid metabolites corresponding to a low level of Porter-Silber chromogen and to an altered coloration of end-products (17). However, from a clinical standpoint, the results with this method have been grossly confirmed by the results of the study on urinary enzymatic extracts.

The determination of urinary corticoids with enzymatic hydrolysis is more accurate, as shown by the results of paper chromatography of these extracts (18, 19). The mean value for total reducing corticoids, determined by this method in 15 normal subjects was 5.7 ± 1.1 mg. in twenty-four hours, with a range of 4.8 mg. (Table 2). Of these alpha-ketolic metabo-

TABLE 2. EXCRETION OF C_{21}-α-KETOL CORTICOIDS (17-HYDROXY AND 17-DESOXY FRACTIONS, AND 17-HYDROXY/17-DESOXY RATIO) IN 15 NORMAL SUBJECTS (ENZYMATIC HYDROLYSIS OF URINE)

Subject	Age (yrs.)	Total reducing corticoids (Folin)* (mg./24 hrs.)		17-Hydroxy‡ (mg./24 hrs.)	17-Desoxy§ (mg./24 hrs.)	Ratio 17-Hydroxy /17-Desoxy
		Total	Corrected†			
1. M	45	5.5	4.7	4.0	0.7	5.7
2. D	30	4.8	4.1	3.8	0.3	12.6
3. F	34	5.2	4.4	4.0	0.4	10.0
4. G	26	6.5	5.5	4.9	0.6	8.1
5. B	31	10.4	8.9	7.8	1.1	7.0
6. C	31	7.9	6.8	5.9	0.9	8.8
7. L	28	9.9	8.5	7.4	1.1	6.7
8. T	52	6.0	5.1	4.7	0.4	11.7
9. Dr	20	4.4	3.7	3.5	0.2	17.0
10. To	30	8.9	7.7	6.3	1.4	4.5
11. Ge	37	6.3	5.4	4.8	0.6	7.0
12. V	21	5.5	4.7	4.3	0.4	9.7
13. P	40	6.6	5.7	4.7	1.0	4.7
14. Tk	26	7.1	6.0	5.7	0.3	19.0
15. A	27	6.7	5.0	3.8	1.2	3.0
		mean: 5.7 ± 1.1		*mean: 5.4 ± 1.05*	*mean: 0.7 ± 0.3*	*mean: 9.2 ± 4.1*

* C_{21}-α-ketol corticoids measured with Folin-Wu phospho-molybdotungstic reagent.

† Corrected value = total value minus 20% of total value (*see* text).

‡ 17-Hydroxycorticoids, catabolites of compound F (cortisol) and eventually compound S (desoxocortisone).

§ Corrected value of total reducing corticoids minus 17-hydroxy = catabolite of compound B (corticosterone) and desoxycorticosterone.

lites, the proportion of 17-hydroxycorticoids was 5.4 ± 1.05 mg. (range, 4.8 mg.), and of 17-desoxycorticoids, 0.7 ± 0.3 mg. (range, 1.2 mg.). The former represents the metabolites of compound F (tetrahydrocortisone and tetrahydrocortisol); the latter represents the metabolites of both compounds B and desoxycorticosterone (mainly tetrahydro-derivatives). Calculation of the ratio of 17-hydroxy to 17-desoxy corticoids yielded high values (9.2 ± 4.1), corroborating a predominance of F metabolites in normal man (Table 2).

Administration of ACTH involves a threefold to fivefold increase of 17-OH excretion. The interpretation of the increase in T.R.C. and 17-desoxycorticoids with the Folin-Wu reaction, during ACTH stimulation, is difficult. Paper chromatography is required for such a determination.

Paper chromatographic determinations were carried out on 3 normal subjects—2 in a basal condition and 1 under stimulation with corticotropin. The results showed that F metabolites are predominant in normal subjects; the ratio of F catabolites to B catabolites was above 5 and increased under stimulation with corticotropin (Table 5).

II. *Chronic fatigue syndrome*

In this group, we studied a) the excretion of total neutral 17-ketosteroids, b) the excretion of corticoids (17-OH and T.R.C.), c) the excretion of alpha-ketolic metabolites, using paper chromatography, and d) the effect of stimulation by corticotropin, using chromatographic techniques.

Neutral 17-ketosteroids

In 31 asthenic male subjects with an average age of 37 years (21 to 54 years), the mean daily excretion was 12.5 mg., the extreme values being 5.0 to 23.5 mg. per twenty-four hours. The values showed a certain degree of scatter and the excretion seemed generally low, compared to that for 30 normal subjects of the same age group (16 mg. per twenty-four hours).

Thus, in asthenia, there was a moderate, but significant decrease of 17-ketosteroids, with a large standard deviation of 5 mg.

We have reported previously considerable variation in the mean 17-ketosteroid excretion in a group of aviation maintenance personnel whose occupation involved tiring work (13.7 ± 4.6 mg. for a mean age of 35 years) (18).

Corticoids

We shall study separately the results with acid hydrolysis and with enzymatic hydrolysis of urine. The behavior of the metabolites of alpha-ketolic corticoids in chronic fatigue is of particular interest, as the changes seem highly significant.

Acid hydrolysis. In 26 of 31 males the daily excretion of 17-hydroxycorticosteroids was decreased. The values ranged from 0.9 mg. to 2.8 mg. per twenty-four hours. In 7 of 9 females, 17-OH excretion was low (Table 3).

The total reducing corticoids (alpha-ketolic side-chain) were decreased in parallel with the 17-OH in only 4 cases (11 per cent). In all other patients (89.9 per cent), T.R.C. remained within normal limits.

The lowest 17-OH values corresponded to normal T.R.C. Normal 17-OH values, although low, corresponded to increased T.R.C. This important dissociation between T.R.C. and 17-OH is evidence that the urine of these subjects contained substances which do not possess a hydroxyl group on C-17.

Table 3 shows the magnitude of the excess of T.R.C. compared to 17-

TABLE 3. EXCRETION OF CORTICOIDS IN CHRONIC ASTHENIA
(ACID HYDROLYSIS OF URINE)

		17-Hydroxy (mg./24 hrs.)	Total reducing corticoids (mg./24 hrs.)	Excess TRC
Male				
	B	1.1	—	—
	B	1.5	—	—
	C	3.1	3.4	0.3
	D	2.8	5.2	2.4
	D	2.0	2.4	0.4
	D	2.3	3.5	1.2
	D	1.7	1.7	0
	G	1.4	1.6	0.2
	G	2.5	5.1	2.6
	F	1.5	4.3	2.8
	H	3.4	5.0	1.6
	H	3.8	—	—
	I	1.7	3.6	1.9
	L	1.7	3.5	1.8
	L	1.7	3.5	1.8
	L	1.2	2.8	1.6
	M	1.9	4.9	3.0
	M	2.6	4.3	1.7
	M	1.7	4.2	2.5
	L	2.7	5.1	2.4
	N	0.9	2.8	1.9
	O	2.0	3.0	1.0
	P	3.5	7.8	4.3
	P	2.9	1.6	—
	P	2.1	3.8	1.7
	P	3.5	7.8	4.3
	S	1.3	2.7	1.4
	S	2.2	3.2	1.0
	S	1.4	4.0	2.6
	S	2.9	5.0	2.1
	S	2.9	5.0	2.1
		mean: 2.19	*mean: 3.95*	*mean: 1.85 ± 0.10*
Female				
	A	1.9	4.3	2.4
	D	2.3	3.9	1.6
	D	2.0	4.5	2.5
	B	2.0	5.2	3.2
	D	2.9	3.9	1.0
	C	2.0	3.1	1.1
	M	2.2	4.2	2.0
	M	1.6	3.8	2.2
	R	1.7	4.9	3.2
		mean: 2.05	*mean: 4.2*	*mean: 2.13*

TABLE 4. EXCRETION OF C_{21}-α-KETOL CORTICOIDS (17-HYDROXY AND 17-DESOXY FRACTIONS, AND 17-HYDROXY/17-DESOXY RATIO) IN 12 ASTHENIC SUBJECTS. (ENZYMATIC HYDROLYSIS OF URINE)

Subject	Age (yrs.)	Total reducing corticoids (Folin)* (mg./24 hr.)		17-Hydroxy‡ (mg./24 hr.)	17-Desoxy§ (mg./24 hrs.)	Ratio 17-Hydroxy /17-Desoxy
		Total	Corrected†			
1. DR	51	11.9	10.7	6.1	4.6	1.3
2. K	30	8.9	8.0	4.8	3.2	1.5
3. P	24	5.9	5.5	2.2	3.3	0.6
4. C	47	6.6	6.4	2.1	4.3	0.5
5. Leg	26	7.4	6.7	3.6	3.1	1.1
6. V	24	11.8	10.7	5.7	5.0	1.1
7. B	36	9.2	8.1	5.5	2.6	2.1
8. R1	42	9.1	8.4	3.9	4.5	0.8
9. R2	42	9.5	8.6	4.5	4.1	1.1
10. Ge	37	13.5	12.6	4.5	8.1	0.5
11. F	27	12.2	10.6	8.0	2.6	3.0
12. G	35	2.0	1.8	1.0	0.8	1.2
		mean: 8.2±2.8		*mean: 4.3±1.7*	*mean: 3.8±1.7*	*mean: 1.2±0.9*

* † ‡ §—*See* footnotes to Table 2.

OH, in sharp contrast to the agreement observed in normal subjects.

Enzymatic hydrolysis. In 12 subjects, the results with alpha-ketolic corticoids by enzymatic hydrolysis confirmed the data of acid hydrolysis (Table 4).

In asthenic subjects, the mean value for T.R.C. was more elevated than in normal subjects (8.2 ± 2.8 mg. per twenty-four hours, with a range of 8.9 mg.). This increase was mostly due to the elevation of the 17-desoxy fraction, the mean value of which was 3.8 ± 1.7 mg. (range, 7.3 mg.). The 17-OH fraction was only slightly decreased (mean, 4.3 ± 1.7 mg., with a range of 7 mg.).

The ratio of 17-hydroxycorticoids to 17-desoxycorticoids is decreased in asthenic subjects, being 1.2 (Table 4) compared to 9.2 (Table 2) in normal subjects. This decrease is strong evidence of a functional adrenal insufficiency in chronic fatigue.

α-Ketolic corticoids determined by paper chromatography

Paper chromatographic determinations of C_{21}-α-ketol steroids were performed in 6 cases of chronic fatigue. The different metabolites were identi-

fied by the migration rate (RDOC), group reactions, and spectra in sulfuric acid. The results of these investigations are listed in Table 5.

Except in Subject 5 (discussed in connection with ACTH stimulation in chronic asthenia), there was a decrease of F catabolites contrasting with an increase of B catabolites.

Functional response of the adrenal cortex to stimulation with corticotropin

The excretion of urinary 17-ketosteroids and corticoids before and after injection of long-acting corticotropin (Cortrophin Z) in 11 of our patients is shown in Table 6. In the twenty-four hours following injection of corticotropin a variable but definite increase of urinary corticoids (17-OH and T.R.C.) occurred. In 8 of the 11 cases an eosinopenia of 90 per cent or more was found; in the remaining 3 cases (Nos. 1, 2 and 8, Table 6) either the

TABLE 5. DETERMINATION OF F AND B CATABOLITES* AND F/B RATIO IN URINE OF NORMAL AND OF ASTHENIC SUBJECTS

Subject	Age (yrs.)	Clinical status	F catabolites (mg./24 hrs.)	B catabolites (mg./24 hrs.)	F/B ratio
1. G	32	Normal	3.4	0.6	5.3
2. S	26	Normal	3.0	0.4	7.2
3. C	45	Normal—ACTH	10.2	1.4	7.2
4. Ge	37	Fatigue	1.1	1.7	0.7
5. R†	45	Fatigue	1.3	0.2	6.5
		—ACTH	7.1	2.3	3.0
6. F	24	Fatigue	3.6	1.3	2.7
		—ACTH	7.2	1.7	4.2
7. V	26	Fatigue+obesity	3.2	1.0	3.2
8. Ch	30	Fatigue	2.6	2.3	1.1
9. P‡	27	Fatigue	3.3	0.8	4.1
		—ACTH	2.2	0.4	5.5
10. LeG	27	Fatigue	1.7	0.7	2.4

* The F (hydrocortisone) and B (corticosterone) catabolites were calculated on the basis of paper chromatography of the extracts obtained from one-fifth to four-fifths of a 24-hour urine (hydrolysis with β-glucuronidase+sulfatase). Recovery of alpha-ketolic metabolites, under our chromatographic conditions, was about two-thirds of total amount.

† There was an abnormal decrease of the F/B ratio after ACTH, but the increase in total 17-OH, determined chemically and by paper chromatography was normal (from 1.3 mg. to 7.1 mg.). The basal values were abnormally low.

‡ There was a decrease of 17-OH during ACTH stimulation—from 3.3 mg. to 2.3 mg. per 24 hours. The F/B ratio was subnormal.

TABLE 6. FUNCTIONAL RESPONSE OF THE ADRENAL CORTEX TO CORTICOTROPIN (ACTH) IN 11 PATIENTS WITH CHRONIC ASTHENIA

Subject and occupation	Age (yrs.)	Diagnosis	Blood eosinophils (per cu. mm.)			Urinary* 17-keto (mg./24 hrs.)		Urinary 17-hydroxy (mg./24 hrs.)		Urinary tot. reducing corticoids (mg./24 hrs.)	
			Bef.†	Aft.‡	Eosinopenia %	Bef.	Aft.	Bef.	Aft.	Bef.	Aft.
1. (C.) Typographer	49	Chronic asthenia; ex-prisoner of war with intestinal ulcer	274	31	88	13.8	15.2	3.1	2.7	3.4	4.5
2. (C.) Employee	41	Chronic asthenia; professional maladaptation			88	9.4	10.0	3.3	3.0	3.2	3.5
3. (G.) Employee	36	Present prisoner of war	73	5	94	8.4	9.8	1.4	1.9	1.6	3.2
4. (H.) No definite work	31	Chronic asthenia; re-signd soldier	429	18	96	15.0	27.0	3.4	5.5	5.0	7.4
5. (L.) Student	21	Recovering from mild tuberculosis	95	2	98	14.3	26.0	1.7	1.9	3.5	5.5
6. (M.) Employee	33	Present prisoner of war				12.5	18.0	2.3	5.8	2.7	8.0
7. (M.) Farmer	49	Chronic asthenia	92	2	97.5	7.3	10.5	1.9	3.4	4.9	6.2
8. (M.) Aviation maintenance	54	Industrial fatigue, overwork, psoriasis	59	17	71	7.5	16.3	2.6	2.9	4.3	8.0
9. (P.) Drawing master	35	Chronic asthenia; present prisoner of war	129	8	93.5	5.5	11.7	2.9	4.1	1.6	3.5
10. (P.) Foundry workman	28	Asthenia with pneumoconiosis	289	2	99	8.0	14.0	3.1	5.3	3.5	6.0
11. (M.) Employee	44	Chronic asthenia with vertigo	127	5	96	14.0	14.0	2.9	3.1	5.0	12.6

* Acid hydrolysis.
† Before ACTH.
‡ After ACTH.

eosinopenia did not reach the level of 90 per cent, or there was only a slight increase of urinary corticoids, especially 17-OH. In all other instances the eosinopenia was clear-cut, indicating adequate stimulation of the adrenal cortex during the first eight hours after injection of corticotropin.

This satisfactory eosinopenic response contrasted with the low excretion of urinary steroids compared to that of normal subjects submitted to the corticotropin test. Furthermore, the 17-OH fraction increased less than the total corticoids, suggesting an increased production of 17-desoxycorticoids (corticosterone) under the influence of corticotropin.

This dissociation of 17-OH and T.R.C., which was already apparent in the asthenic patients prior to stimulation with corticotropin, became more evident after stimulation.

In addition to the determination of C_{21}-α-ketol metabolites, using acid

TABLE 7. DETERMINATION OF URINARY 17-OH-CORTICOIDS (ENZYMATIC HYDROLYSIS) IN NORMAL AND IN ASTHENIC SUBJECTS UNDER STIMULATION WITH LONG-ACTING CORTICOTROPIN (ACTH)

Subject	Diagnosis	Urinary 17-OH-corticoids (mg./24 hrs.)	
		Before ACTH	After ACTH
1. M	Normal	3.5	14.3
2. K.	Normal	4.8	28.8
3. B.	Normal	3.9	10.7
4. R.	Normal	3.9	19.6
5. Ro	Normal	6.2	24.9
6. Reb.	Anorexia	2.4	8.5
7. G.	Normal	2.1	14.2
8. P.	Anorexia	2.2	12.6
9. C.	Anorexia	2.1	17.4
10. Roc.*	Chronic asthenia	4.5	7.0
11. F.	Chronic asthenia	8.0	12.0
12. M.	Chronic asthenia	3.1	7.5
13. S.	Chronic asthenia	3.3	4.4
14. Fa.	Chronic asthenia	3.6	7.1
15. Pi†	Chronic asthenia	3.7	2.2

* Case 10 corresponds to Case 5, Table 5. Paper chromatography in this case showed a decrease of the urinary F/B ratio after ACTH.

† Case 15 corresponds to Case 9, Table 5. There was a decrease in 17-OH during stimulation with ACTH, although the F/B ratio was normal.

hydrolysis, we studied the magnitude of 17-OH excretion following administration of corticotropin to normal and to asthenic subjects, using enzymatic hydrolysis. These results are listed in Table 7.

The pattern of 17-OH excretion under ACTH stimulation in asthenic subjects pointed to a defect of production of cortisol by the adrenals. Chromatographic studies demonstrated an increase in the relative excretion of corticosterone (or dehydro-corticosterone) catabolites. In Case 10, in which the F/B ratio was normal prior to corticotropin, adrenal stimulation with corticotropin induced a decrease in this ratio, and a marked excretion of 17-desoxy catabolites.

In normal subjects the increase in 17-OH corticoids after administration of corticotropin ranged between 174 and 747 per cent; in the cases of chronic asthenia, this increase ranged between 0 and 142 per cent.

DISCUSSION

This study of urinary corticoids (17-keto and α-ketolic) throws some

new light on chronic fatigue. The lack of gross disturbances of the adrenals, points to a dysfunction rather than a total impairment.

Cortisol (compound F) and corticosterone (compound B) have been found in adrenal venous blood and in peripheral blood, and are quantitatively the most important part of adrenocortical secretion. The F/B ratio may present some variations.

Romanoff *et al.* (19) have shown a predominant secretion of compound F in adrenalectomized patients with cancer, and during stimulation with corticotropin (ratio F/B = 10). Sweat (20) related variable amounts of compound B in peripheral venous blood with a predominance of compound F (F/B ratio, 2.5 to 6.3), as previously reported by Cope and Hurlock (21).

Paper chromatography of extracts of enzymatically hydrolyzed urines yields evidence of steroids related to corticosterone—allo-pregnane-3α, 11β, 21-triol-20-one (allo-tetrahydro B); pregnane-3α,11β,21-triol-20-one (tetrahydro B); and pregnane 3α,21-diol-20-dione (tetrahydro A) (22–26). Their amount is very low compared with that of cortisol metabolites (tetrahydro-cortisone and tetrahydrocortisol), the ratio being grossly F/B = 5. Our data are in good agreement with those previously reported.

The role of corticoids in chronic fatigue requires some further comment. The moderate decrease in urinary neutral 17-ketosteroids does not furnish an adequate indication of the degree of adrenal dysfunction, since the 17-ketosteroids are a heterogenous group of catabolites of testicular and adrenal androgens, and 17-OH corticoids.

Our study of 17α-ketol, C_{21} urinary corticoids (with or without 17α-hydroxyl), using different techniques (chemical measurements after acid and enzymatic hydrolysis, and paper chromatography), provides better evidence of a specific adrenal impairment in chronic fatigue. Our first studies in 40 asthenics (Table 3) show a lack of correlation between α-ketol catabolites of cortisol and corticosterone. More accurate methods, using enzymatic hydrolysis, comparative studies of Porter-Silber chromogens and reducing power with Folin-Wu reagent (9) or blue tetrazolium (28), show a definite increase in the excretion of 17-desoxy compounds compared with 17-hydroxy compounds. The urinary 17-hydroxy/17 desoxy ratio, which is 9.2 in normal man, is decreased in asthenic subjects (1, 2)—representing an excess of corticosterone and eventually desoxycorticosterone catabolites.

Results of paper chromatography furnish further confirmation, showing increased amounts of tetrahydro-B, allotetrahydro-B and tetrahydro-A in chronic fatigue.

Stimulation with corticotropin in this condition does not increase the F/B ratio as compared with normal man. The 17-OH fraction is increased only slightly, whereas the B catabolites are magnified. Thus the basic dis-

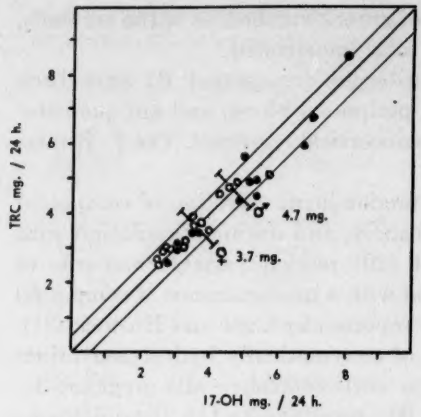

FIG. 1. 17-Hydroxycorticoids and total reducing corticoids in normal men and women. The values for 17-OH and TRC are roughly similar. The mean value for corticoids is lower in women (circles) than in men (dots). (Acid hydrolysis of urine.)

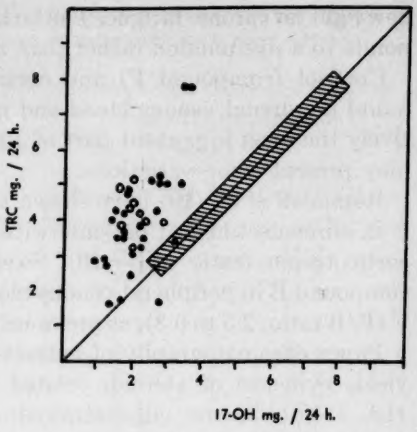

FIG. 2. 17-Hydroxycorticoids and total reducing corticoids in 36 cases of chronic asthenia. There was significant dissociation between TRC and 17-OH due to the presence of an excess of 17-desoxycorticoids, metabolites of corticosterone. (Acid hydrolysis of urine.)

crepancy between B and F catabolites is exaggerated by administration of corticotropin in chronic asthenia, as shown in Figures 1–3.

This evidence suggests an alteration of adrenal function in chronic asthenia. Several authors cited by Hechter and Pincus (2) have demon-

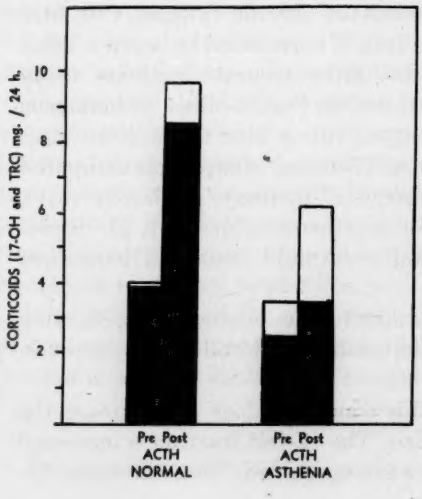

FIG. 3. Effect of 40 USP units of zinc corticotropin in normal (left) and in asthenic subjects (right). The black columns represent 17-OH-corticoids and the white columns, total reducing corticoids. (Acid hydrolysis of urine.)

strated an enzymatic process in the adrenal cortex which elaborates the cortical hormones. In human adrenals, the trend toward a predominant production of 17-hydroxycorticosterone seems to be localized at the level of the intermediary link of progesterone; an enzyme then hydroxylates C-17. If this hydroxylation does not occur, the biosynthesis continues toward corticosterone. In normal conditions, only a small portion of the metabolites seems to escape this 17-hydroxylation.

In chronic asthenia the procedure apparently is different. The occurrence of a considerable amount of 17-desoxy metabolites in basic conditions, and the even greater amount after stimulation by corticotropin, shows deficient 17-hydroxylation in the adrenal cortex. This alteration of cortical secretion may be ascribed to a disturbance in the enzymatic process. One might compare this disturbance of 17-hydroxylation to the alteration which takes place in the 21-hydroxylation in congenital adrenal hyperplasia, as reported by Bongiovanni *et al.* (30). Yet there is a difference between these two procedures. Whereas 17-hydroxylation may be skipped and hormonal biosynthesis occur through the pathway of corticosterone, no such exit exists for 21-hydroxylation.

Repeated stress in asthenic subjects may thus involve a decrease of the adrenocortical power of 17-hydroxylation, producing a steroid with glucocorticoid and anti-inflammatory properties clearly inferior to those of cortisol (31). This may explain certain symptoms of chronic asthenia, such as continual "tiredness" and the tendency to hypoglycemia.

SUMMARY

Study of the excretion of urinary corticoids in chronic asthenia under basal conditions and under the influence of long-acting corticotropin has yielded the following data:

1. Urinary 17-ketosteroid excretion is subnormal; the decrease is moderate and does not attain the level usually observed in organic adrenocortical failure.

2. Excretion of C_{21},17-desoxycorticoids is relatively increased, suggesting an alteration in the enzymatic process of 17-hydroxylation in the adrenals of these subjects.

3. This disturbance of cortical biosynthesis seems to induce an increased secretion of corticosterone. The increased secretion of this particular corticoid instead of hydrocortisone may explain the disturbances which characterize chronic asthenia, as corticosterone has less glucocorticoid activity.

4. In asthenic subjects, stimulation with corticotropin produces only a slight increase in 17-ketosteroids and corticoids. Furthermore, this stimulation accentuates the dissociation between the decreased amounts of 17-hydroxycorticoids and the excess amounts of 17-desoxycorticoids.

5. Consequently, there is a condition of moderate adrenocortical insufficiency in chronic asthenia, which is of functional nature and due to a disturbance in the process of 17-hydroxylation.

REFERENCES

1. Pincus, G.: Aspects du métabolisme des stéroïdes hormonaux, *in* Actualités Biochimiques. Paris, Liège, Masson-Desoer, 1955.
2. Hechter, O., and Pincus, G.: Genesis of the adrenocortical secretion, *Physiol. Rev.* **34**: 459, 1954.
3. Albeaux-Fernet, M., and Romani, J. D.: La fonction surrénalo-testiculaire dans les cirrhoses alcooliques, *Presse méd.* **64**: 43, 1956.
4. Romani, J. D.: Les métabolites urinaires des corticoïdes chez l'homme. Les états d'insuffisance fonctionnelle de la secrétion corticale, *Compt. rend. Soc. de biol.* **149**: 1906, 1955.
5. Albeaux-Fernet, M.; Orabona, M. L., and Romani, J. D.: L'exploration fonctionnelle du cortex surrénal au cours des affections hépatiques, *Semaine hôp. de Paris* **32**: 70, 1956.
6. Callow, N. H., and Callow, R. K.: Isolation of 17-ketosteroids from urine of normal women, *Biochem. J.* **33**: 931, 1939.
7. Jayle, M. F., and Crepy, O.: Evaluation des 3-alpha et des 3-beta steroïdes neutres, *Ann. d'endocrinol.* **14**: 74, 1953.
8. Romani, J. D.: Une méthode de dosage des glyco-corticoïdes dans les urines. Technique et résultats, *Semaine hôp. de Paris* **31**: 3154, 1955.
9. Romani, J. D.; Bugard, P., and Fisher, G.: Étude de l'élimination urinaire des corticoïdes alpha-cétoliques chez le sujet normal et au cours de la fatigue chronique. Evaluation après hydrolyse enzymatique des urines, *Compt. rend. Soc. de biol.* (to be published).
10. Porter, C. C., and Silber, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201, 1950.
11. Querido, A.; Goslings, J.; Cats, A., and Kassenaar, A.: Clinical evaluation of corticotropin, *J. Clin. Endocrinol. & Metab.* **14**: 681, 1954.
12. den Oudsten, S. A.; van Leeuwen, L., and Coers, R. J.: Corticotropin-zinc: clinical observations on an ACTH preparation with prolonged action, *J. Clin. Endocrinol. & Metab.* **14**: 680, 1954.
13. Zaffaroni, A.: Micromethods for the analysis of adrenocortical steroids, *Recent Progr. Hormone Res.* **8**: 51, 1953.
14. Romani, J. D.: Etude des métabolites urinaires des corticoïdes chez l'homme. Identification des catabolites alpha-cétoliques dans les urines de 24 h., *Compt. rend. Soc. de biol.* **150**: 644, 1956.
15. Romani, J. D.: Étude des métabolites urinaires des corticoïdes chez l'homme. Données quantitatives, *Compt. rend. Soc. de biol.* **150**: 887, 1956.
16. Romani, J. D.: Technique d'étude des métabolites des corticoïdes chez l'homme. I. Méthode de détermination et résultats chez le sujet normal, *Compt. rend. Soc. de biol.* **149**: 1751, 1955.
17. Romani, J. D.: Unpublished.
18. Albeaux-Fernet, M.; Bellot, L.; Bugard, P.; Deribreux, J.; Funck-Brentano, L.; Gélinet, M., and Romani, J. D.: Fatigue et endocrinologie, *L'Année Endocrinologique* **6**: 18, 1954.
19. Romanoff, E. B.; Hudson, P., and Pincus, G.: Isolation of hydrocortisone and

corticosterone from human adrenal vein blood, *J. Clin. Endocrinol. & Metab.* **13:** 1546, 1953.

20. Sweat, M. L.: Adrenocorticosteroids in peripheral and adrenal venous blood of man, *J. Clin. Endocrinol. & Metab.* **15:** 1043, 1955.
21. Cope, C. L., and Hurlock, B.: Some aspects of adrenal cortical metabolism, *Clin. Sc.* **13:** 69, 1953.
22. Engel, L. L.; Carter, P., and Springer, M. J.: Isolation from urine of ketonic metabolites of administered corticosterone, *Fed. Proc.* **13:** 204, 1954.
23. Touchstone, J. C.; Bulaschenko, H.; Richardson, E. M., and Dohan, F. C.: The isolation of pregnane-3α, 11β, 21-triol-20-one, allo-pregnane-3α, 11β, 21-triol-20 one, pregnane-3α,21-diol-11,20-dione and Δ^4-pregnene-11β,21-diol-3,20-dione from the urine of normal subjects treated with corticotropin, *Arch. Biochem. & Biophys.* **52:** 284, 1954.
24. Engel, L. L.; Carter, P., and Fielding, L. L.: Urinary metabolites of administered corticosterone. I. Steroid liberated by glucuronidase hydrolysis, *J. Biol. Chem.* **213:** 99, 1955.
25. Richardson, E. M.; Touchstone, J. C., and Dohan, F. C.: Urinary alpha-ketolic steroid metabolites of cortical hormones administered to subjects with adrenal cortical insufficiency, *J. Clin. Invest.* **34:** 285, 1955.
26. Dohan, F. C.; Touchstone, J. C., and Richardson, E. M.: The effect of ACTH and pathological increases in adrenal cortical function on urinary alpha-ketolic steroid metabolites, *J. Clin. Invest.* **34:** 485, 1955.
27. Albeaux-Fernet, M.; Bugard, P., and Romani, J. D.: Étude de l'élimination des corticoïdes urinaires au cours des états de fatigue chronique, *Ann. d'endocrinol.* **17:** 220, 1956.
28. Romani, J. D.: Unpublished.
29. Dorfman, R. I.: *In vivo* metabolism of neutral steroid hormones, *J. Clin. Endocrinol. & Metab.* **14:** 318, 1954.
30. Bongiovanni, A. M.; Eberlein, W. R., and Cara, J.: Studies on the metabolism of adrenal steroids in the adrenogenital syndrome, *J. Clin. Endocrinol. & Metab.* **14:** 409, 1954.
31. Hechter, O.: The adrenocortical secretory products *in* The Mechanism of Inflammation. International Symposium. Montréal, Canada, Acta Inc., Med. Publ., 1953, p. 21.

THE CONJUGATED 17-KETOSTEROIDS OF HUMAN URINE*

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IT IS generally recognized that the steroids of human urine are principally conjugated to sulfuric acid and to glucuronic acid. Although the urinary corticosteroids (such as the metabolites of cortisol and corticosterone) are for the most part quantitatively released from their water-soluble forms of conjugation by incubation with various preparations of β-glucuronidase (1), the urinary 17-ketosteroids are not. Only 40 to 65 per cent of the total 17-ketosteroids of normal urine are hydrolyzed by β-glucuronidase (2–5).

We have directed our attention to the examination of the individual 17-ketosteroids released enzymatically from human urine. This has been accomplished by means of chromatography on paper followed by elution of the individual steroids and their quantitative measurement by the Zimmermann reaction.

EXPERIMENTAL

Normal male urine

A. Varying amounts of β-glucuronidase[1] were added to 25-ml. portions of a pooled male urine specimen after adjusting to pH 5, and the urines incubated at 37° C. for forty-eight hours. The 17-ketosteroids were extracted with ether-chloroform (4:1) (6) and determined by the Wilson micro-modification of the Callow method (7). The results (Table 1) show that a minimum of 50 units of liver β-glucuronidase per ml. of urine was necessary for maximum release of 17-ketosteroids.

B. A 250-ml. portion of the urine was incubated with β-glucuronidase (100 units per ml. of urine) as described. After forty-eight hours the mixture was extracted with ether-chloroform (4:1). The extract was washed with cold sodium hydroxide (0.1N) and

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[1] The enzyme preparation used throughout this study was a brand of beef liver β-glucuronidase named Ketodase (Warner-Chilcott Laboratories, New York). This preparation was found during this study to contain no steroid sulfatase when tested with sodium dehydroepiandrosterone sulfate as substrate.

TABLE 1. 17-KETOSTEROIDS OF NORMAL MALE URINE RELEASED BY β-GLUCURONIDASE

(25 ml. of urine (pH 5.0) incubated at 37° C. for forty-eight hours and extracted with ether-chloroform (4:1)

β-Glucuronidase (units/ml.)	17-Ketosteroids (μg. per 25 ml.)
10	185, 204 (195)
25	286, 298 (292)
50	474, 325 (400)
100	405, 450 (427)

treated with Girard's reagent T to provide a neutral ketonic fraction which was subjected to quantitative paper chromatography in the ligroin-propylene glycol system (8) as described by Rubin *et al.* (9). The individual 17-ketosteroid zones were eluted and the 17-ketosteroid content of each determined by the Wilson method (7). The values obtained are listed in Table 2. The identity of each steroid was verified by absorption spectra in sulfuric acid (10).

C. One liter of the pooled urine was adjusted to pH 1.0, 150 ml. of concentrated hydrochloric acid added, and the mixture refluxed with 250 ml. of carbon tetrachloride

TABLE 2. 17-KETOSTEROIDS OF NORMAL MALE URINE (POOL)

Steroid	17-KS (mg./liter)*		Per cent β-gluc. recovery in terms of HCl-CCl_4 recovery
	Hydrolysis with HCl-CCl_4†	Hydrolysis with β-glucuronidase‡	
Total ketonic fraction§	19.80	8.4	42
Androsterone	5.10	1.04	20
11-Ketoandrosterone	0.34	0.08	23
11-Hydroxyandrosterone	—	0.15	55
as 9,(11)-androstenolone‖	0.27	none	
Etiocholanolone	5.55	3.84	69
11-Ketoetiocholanolone	1.09	0.87	80
11-Hydroxyetiocholanolone	—	0.27	93
as 9,(11)-etiocholanolone‖	0.29	none	
Total recovered	*12.68 (64%)*	*5.80 (69%)*	

* Determined by Wilson micro-modification (7) of the Zimmermann reaction.

† Fifteen volumes of conc. HCl per 100 ml. of urine, refluxed under CCl_4 for twelve hours; CCl_4 replaced with fresh solvent and urine refluxed for additional twelve hours.

‡ Urine treated with liver β-glucuronidase preparation, 100 units per ml., at pH 5.0 and 37° C. for twenty-four hours.

§ Ketonic fraction prepared from total neutral extract by Girard reagent T.

‖ The values were determined spectrophotometrically, and are maximum amounts.

for six hours. The carbon tetrachloride was then replaced with another 250-ml. portion, and the mixture was again refluxed (9). The combined extracts were evaporated and the procedure described in paragraph B was carried out. Table 2 presents the results of chromatographic and colorimetric determinations of the individual 17-ketosteroids following acid hydrolysis. The estimation of the amounts of 9,(11)-dehydro derivatives of androsterone and etiocholanolone (resulting from the action of mineral acid on 11β-hydroxyandrosterone and etiocholanolone) present in the androsterone and etiocholanolone zones (8) was made by means of sulfuric-acid chromogen spectra (Savard, unpublished).

D. The data recorded in Tables 3 and 4 were obtained by the following procedures: a) total 17-ketosteroids were determined on aliquots of 24-hour specimens by the method of Drekter (11), and b) 100 ml. of each specimen was adjusted to pH 5.0, incubated with β-glucuronidase (100 units per ml.) for forty-eight hours at 37° C., and extracted with dichloromethane. The dried extract was evaporated to dryness under reduced pressure and transferred to a 1.0-ml. volumetric flask. Four 100-μl. (0.1 ml.) aliquots of the solution were chromatographed on filter paper. After development for twenty-four hours and drying, two of the strips were used for location and characterization ("mixed" chromatogram) of the zones of androsterone and etiocholanolone; the corresponding areas of the other two strips were eluted and their 17-ketosteroid content determined quantitatively by the Zimmermann reaction (12). Additional verification of the nature of the steroids eluted was made by means of the sulfuric-acid chromogens.

RESULTS AND DISCUSSION

The results of Experiment B, shown in Table 2, indicate that β-glucuronidase releases less than half of the total 17-ketosteroids present in human male urine. This is in agreement with the data of Glenn and Nelson, who used the same liver enzyme preparation (2), as well as with the results of others who used enzymes of bacterial (3, 4) or splenic (5) origin. Results of analysis of the 6 steroids (Table 2) show that incomplete hydrolysis of the 17-ketosteroid conjugates is not confined to any single individual steroid or group of steroids, but is incomplete for all 6 investigated; the seventh important urinary 17-ketosteroid, dehydroepiandrosterone, has not been observed by the authors in extracts of β-glucuronidase-treated urine (13). The quantities of androsterone and of 11-keto- and 11β-hydroxyandrosterones obtained following enzymic treatment of the urine were only 20 to 55 per cent of the amounts obtained following acid hydrolysis, whereas the amounts of etiocholanolone, 11-keto- and 11β-hydroxyetiocholanolone were between 69 and 93 per cent of the acid-treatment values. The implication to be drawn therefore is that these 6 steroids are only in part conjugated to glucuronic acid.[2] Since additional treatment of this and other urines with a second amount of β-glucuronidase failed to yield additional

[2] The conclusion drawn here is in the tradition of current thought in this field (1, 14, 15) and should be considered contingent upon more direct evidence such as isolation or demonstration by paper chromatography (24) of the steroid conjugates.

TABLE 3. AMOUNTS OF ANDROSTERONE AND ETIOCHOLANOLONE (MG. PER 24 HRS.) RELEASED BY β-GLUCURONIDASE HYDROLYSIS OF URINE IN GROUPS OF NORMAL AND DISEASED SUBJECTS

	Total 17-KS*	Androsterone		Etiocholanolone		Ratio E/A	Pathology	Rx
		Wt.	%17-KS	Wt.	%17-KS			
				Normal males				
1	7.1	0.42	5.9	1.6	22.4	3.8		
2	11.0	0.94	8.4	1.5	13.5	1.6		
3	5.3	0.95	17.9	2.2	41.5	2.3		
4	8.3	0.44	5.3	1.4	16.8	3.2		
Mean ± S.D.		*0.69± .26*		*1.7± .5*				
				Normal females				
1	4.3	0.65	15.2	1.3	30.4	2.0		
2	22.0	1.70	7.7	6.8	30.9	4.0		
3	8.2	0.53	6.5	2.9	35.0	5.5		
4	11.2	0.81	7.2	2.5	22.2	3.1		
5	11.2	0.30	2.7	1.5	13.3	5.0		
6	13.3	1.50	11.3	4.4	33.0	2.9		
7	11.1	0.61	5.5	2.9	26.1	4.8		
8	7.5	0.64	8.6	2.5	33.3	3.8		
9	9.1	0.80	8.8	1.7	18.7	2.1		
10	11.0	1.46	13.5	2.0	18.8	1.4		
Mean ± S.D.		*.90± .49*		*2.8±1.6*				
Mean+S.D. (m. & f.)		*.84± .41*	*8.9±4.0*	*2.5±1.4*	*25.4±8.4*	*3.5±1.4*		
				Diseased males				
1	4.7	0.39	8.3	0.79	16.9	2.1	Asthma	
2	2.2	0.48	21.8	1.10	50.0	2.3	Asthma	
				Diseased females				
1	4.4	0.27	6.1	1.80	41.0	6.6	Asthma	
2	9.5	1.10	11.6	2.20	23.1	2.0	Asthma	Cortisol
3	3.6	0.20	5.6	0.77	21.4	3.8	Asthma	
4	0.8	0.02	2.5	0.09	11.4	4.5	Basal-cell carcinoma	
5	3.9	0.41	10.5	1.10	28.2	2.7	Hypertension	
6	1.7	0.49	28.8	0.65	38.2	1.3	Polyarthritis	
7	5.6	0.10	1.8	1.10	19.7	11.0	Breast cancer	Thyroid; cortisone
8	7.0	0.11	1.6	0.31	4.4	2.8	Breast cancer	Thyroid; cortisone
9	32.0	1.80	5.6	10.90	34.0	6.0	Cushing's synd.	
10	4.6	0.38	8.3	0.96	21.0	2.5		
11	1.4	0.15	10.7	0.13	9.3	0.8	Breast cancer	
Mean± S.D. (m. & f.)			*9.5±7.6*		*24.5±12.8*	*3.7±0.8*		

* Determined by the Drekter method (11).

† Calculated for the combined values for males and females.

amounts of 17-ketosteroid, it is apparent that these steroids remain unextractable in other conjugated forms, most likely as sulfates (14, 15). This well recognized point is borne out in the recently successful use of combined β-glucuronidase and steroid sulfatase preparations of molluscular origin which are effective in releasing most if not all of the 17-ketosteroids of urine (16–18). The present study, in showing this apparent dual form of conjugation of androsterone and its 11-keto and 11β-hydroxy derivatives, and of etiocholanolone and 11-keto and 11β-hydroxyetiocholanolone, parallels the findings of Cohen and Bates in the case of the estrogens of human urine (19).

The 24-hour excretion levels of androsterone are 3.36 ±0.57 mg. in males and 3.80 ±0.59 mg. in females; the 24-hour value for etiocholanolone is 3.89 ±0.59 mg. in males and 3.05 ±0.56 mg. in females (9, 20, 21). In directing our attention particularly to the amounts of these two major components of the urinary 17-ketosteroids released enzymatically from the urines of human subjects, it is apparent from Tables 3 and 4 that only a fraction of the totals are hydrolyzed. Among the normal subjects aged 20–44 years, the average amount of androsterone obtained by enzymatic

TABLE 4. AMOUNTS OF ANDROSTERONE AND ETIOCHOLANOLONE (MG. PER 24 HRS.) RELEASED BY β-GLUCURONIDASE HYDROLYSIS OF URINE IN 3 SUBJECTS

Subject	Total 17-KS*	Androsterone		Etiocholanolone		Ratio E/A
		Wt.	% 17-KS	Wt.	% 17-KS	
1 (M)	7.1	0.42	5.9	1.6	22.4	3.8
	6.4	0.39	6.1	1.3	20.4	3.3
	6.5	0.47	7.2	1.8	27.6	3.8
	10.4	0.78	7.5	1.9	18.2	2.4
	8.0	0.66	8.3	1.7	21.2	2.6
	6.6	0.36	5.4	1.7	25.8	4.7
2 (F)	9.1	0.80	8.8	1.7	18.7	2.1
	9.0	0.46	5.1	1.2	13.2	2.6
	6.1	0.30	4.9	0.9	14.7	3.0
	7.0	1.20	17.5	2.3	33.5	1.9
	8.3	1.30	15.9	3.0	36.2	2.3
	7.3	1.50	20.5	2.0	27.4	1.3
3 (F)	11.0	1.46	13.5	2.0	18.8	1.4
	6.6	1.10	16.8	2.4	36.4	2.2
	8.8	0.88	10.0	2.4	27.3	2.7
	8.7	1.90	22.0	4.1	47.4	2.1
Average		*0.87*	*11.0*	*2.0*	*25.5*	*2.6*

* Determined by Drekter method (11).

treatment was 0.84 ± 0.41 (range, 0.42 to 1.70) mg. per twenty-four hours, and the amount of etiocholanolone was 2.5 ± 1.4 (range, 1.3 to 6.8) mg. per twenty-four hours. The values in the few diseased people who were studied fell, for the most part, within the foregoing ranges. The overall picture in normal males and females and in sick people (male and female) presented an arresting regularity. Androsterone values were respectively 8.9 ± 0.40 and 9.5 ± 7.6 (range 1.6 to 28.0) per cent of the total 17-ketosteroid level. Etiocholanolone values were respectively 25.4 ± 8.4 and 24.5 ± 12.8 (range 4.4 to 41.5) per cent of the total. Among the individual subjects studied for more than a single day (Table 4) the values were reasonably constant.

A point of extreme interest in the results of this study is the consistently higher amount of etiocholanolone than androsterone that is released by β-glucuronidase (13). When the absolute amounts (in mg.) of these two steroids are expressed as the ratio etiocholanolone/androsterone (E/A) per 24 hours (Tables 3 and 4), the excess of the former over the latter is reflected in the ratio being always greater than 1 (except in the single case of advanced cancer, Table 3). Normal people, both males and females, show a similar ratio of 3.5 ± 1.4. Furthermore the E/A ratio varies only slightly from day to day in single individuals studied (Table 4). Similarly, greater amounts of 11-keto and 11β-hydroxyetiocholanolone than those of the corresponding oxygenated derivatives of androsterone are obtained after enzyme treatment (Table 2); here the E/A ratio is 4.9. The data point to etiocholanolone and 11-keto-11β-hydroxyetiocholanolones being conjugated to glucuronic acid to a much greater extent than androsterone and 11-keto and 11β-hydroxyandrosterones. A plausible explanation of this phenomenon lies in a consideration of the origin of the urinary derivatives of etiocholanolone. In human metabolism, in contrast to androsterone (5α) and its derivatives, etiocholanolone derivatives (5β) are formed directly from *both* C_{19} and C_{21} precursors (22). The latter are almost exclusively conjugated to glucuronic acid (14) and in the course of transformation to 17-ketosteroids of the etiocholane (5β) series (22), the acid of conjugation remains unchanged. Supporting this concept is the fact that β-glucuronidase (liver) releases more than 90 per cent of urinary 17-ketosteroids consisting almost totally of 11-keto and 11β-hydroxyetiocholanolone (resulting from exogenously administered corticosteroids, tetrahydrocortisol (23) and cortisone (15).

SUMMARY

Incubation with β-glucuronidase releases from urine only a fraction of the total amounts present of androsterone, 11-ketoandrosterone, 11β-hydroxyandrosterone, etiocholanolone, 11-ketoetiocholanolone, and 11β-

hydroxyetiocholanolone. A significantly greater proportion of the derivatives of etiocholanolone than of androsterone is released by the enzymatic treatment.

REFERENCES

1. Mason, H. L.: Hydrolysis of conjugates and extraction of urinary corticosteroids, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press, Inc., 1954, vol. 9, p. 267.
2. Glenn, E. M., and Nelson, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13**: 911, 1953.
3. Buehler, H. J.; Katzman, P. A.; Doisy, P. P., and Doisy, E. A.: Hydrolysis of conjugated steroids by bacterial glucuronidase, *Proc. Soc. Exper. Biol. & Med.* **72**: 297, 1949.
4. Buehler, H. J.; Katzman, P. A., and Doisy, E. A.: Hydrolysis of conjugates of neutral ketosteroids, *Proc. Soc. Exper. Biol. & Med.* **78**: 3, 1951.
5. Cohen, S. L.: The hydrolysis of steroid glucuronides with calf spleen glucuronidase, *J. Biol. Chem.* **192**: 147, 1951.
6. Heard, R. D. H., and Sobel, H.: Steroids. VIII. A colorimetric method for the estimation of reducing steroids, *J. Biol. Chem.* **165**: 699, 1946.
7. Wilson, H.: Chromogenic values of various ketosteroids in a micro modification of the Zimmermann reaction: comparison with the macro procedure, *Arch. Biochem.* **52**: 217, 1954.
8. Savard, K.: Paper partition chromatography of C_{19} and C_{21}-ketosteroids, *J. Biol. Chem.* **202**: 457, 1953.
9. Rubin, B. L.; Dorfman, R. I., and Pincus, G.: Quantitative determination of seven urinary 17-ketosteroids, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press, Inc., 1954, vol. 9, p. 213.
10. Bernstein, S., and Lenhard, R. H.: The absorption spectra of steroids in concentrated sulfuric acid. I. Methods and data, *J. Org. Chem.* **18**: 1146, 1953.
11. Drekter, I. J.; Heisler, A.; Scism, G. R.; Stern, S.; Pearson, S., and McGavack, T. H.: The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids, *J. Clin. Endocrinol. & Metab.* **12**: 55, 1952.
12. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determination of substances containing the grouping $—CH_2 \cdot CO—$ in urine extracts as an indication of androgen content, *Biochem. J.* **32**: 1312, 1938.
13. Birke, G., and Plantin, L. O.: Studies on urinary 17-ketosteroids. Isolation and determination of the 17-ketosteroids in urine, *Acta med. scandinav.* **148**: (Suppl. 291) 1-95, 1954.
14. Lieberman, S.; Mond, B., and Smyles, E.: Hydrolysis of urinary ketosteroid conjugates, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press, Inc., 1954, vol. 9, p. 113.
15. Gallagher, T. F.; Bradlow, H. L.; Fukushima, D. K.; Beer, C. T.; Kritchevsky, T. H.; Stokem, M.; Eidinoff, M. L.; Hellman, L., and Dobriner, K.: Studies of the metabolites of isotopic steroid hormones in man, *Ibid.*, p. 411.

16. HENRY, R., and THEVENET, M.: Action du suc digestif fluid d'Helix pomatia (L.) sur les différents stéroides conjugués urinaires, *Bull. Soc. chim. biol.* **34:** 886, 1952.
17. STITCH, S. R., and HALKERSTON, I. D. K.: Enzymatic hydrolysis of urinary neutral 17-ketosteroid conjugates, *Nature* **172:** 398, 1953.
18. JAYLE, M. F., and BAULIEU, E. E.: Données expérimentales sur l'hydrolyse des 17-cétostéroides conjugués urinaires par les diastases du suc digestif d'Helix pomatia (L.), *Bull. Soc. chim. biol.* **36:** 1391, 1954.
19. COHEN, H., and BATES, R. W.: Occurrence and determination of conjugated sulfates of estrogens in urine from pregnant women, *Endocrinology* **45:** 86, 1949.
20. DOBRINER, K.; KAPPAS, A.; RHOADS, C. P., and GALLAGHER, T. F.: Studies in steroid metabolism. XIX. The α-ketosteroid excretion pattern in normal males, *J. Clin. Invest.* **32:** 940, 1953.
21. KAPPAS, A., and GALLAGHER, T. F.: Studies in steroid metabolism. XXXVIII. The α-ketosteroid excretion pattern in normal females and the response to ACTH, *J. Clin. Invest.* **34:** 1566, 1955.
22. DORFMAN, R. I., and UNGAR, F.: Metabolism of Steroid Hormones. Minneapolis, Burgess Publishing Co., 1953.
23. SAVARD, K.; GOLDFADEN, S. H., and DORFMAN, R. I.: The *in vivo* metabolism of dihydrocortisol and tetrahydrocortisol in human subjects, *Fed. Proc.* **13:** 228, 1954.
24. LEWBART, M. L., and SCHNEIDER, J. J.: Paper chromatography of steroid glucuronosides and sulphates, *Nature* **176:** 1175, 1955.

CORTISONE POTENTIATION OF THE HYPERGLYCEMIC ACTION OF INSULIN PREPARATIONS

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IN A previous study the authors (1) demonstrated that in rabbits pretreated with cortisone there is a marked initial rise of the blood sugar level after large intravenous doses of commercial regular insulin. Such insulin contains glucagon. The hyperglycemic effect was thought to be due to the action of this glucagon on the greatly increased level of liver glycogen induced by the administration of cortisone. These experiments demonstrated further that the extent and duration of the hyperglycemia varied directly with the insulin dosage, up to a maximum obtained with 100 units. Regarding hypoglycemia, on the other hand, it was noted that the extent was approximately the same, whether the insulin dosage was 10 units or 500 units. However, the nadir appeared much earlier when lower dosages were given, so that 10 units of insulin appeared to have the greatest hypoglycemic effect. These observations were interpreted as signifying that the response of the organism to insulin becomes maximal at relatively low dosage levels, and that the glucagon present in regular insulin delays rather than inhibits the appearance of hypoglycemia. These findings were not in agreement with those of Root *et al.* (2), who reported that glucagon added to insulin does not affect the assay of insulin by either the mouse or the rabbit method. This discrepancy, it was felt, was due to the increased responsivity of the cortisone-pretreated animal to the hyperglycemic action of the glucagon contained in the large amounts of insulin which were administered.

The present study was undertaken to verify the hypothesis that cortisone through its hepatic glycogenic effect potentiates the action of glucagon, and to investigate further the effects of glucagon and insulin on the blood sugar levels of cortisone-pretreated rabbits. For this purpose, glucagon alone and in combination with various amounts of glucagon-free insulin was injected intravenously into normal and cortisone-pretreated rab-

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bits, and large doses of commercial regular insulin were administered to alloxan diabetic rabbits. Furthermore, an attempt was made to determine whether subcutaneously administered regular insulin or protamine zinc insulin also induced a hyperglycemic response after pretreatment of the animal with cortisone.

MATERIAL AND METHODS

The study was carried out on 270 normal male and female rabbits, each weighing between 2500 and 3000 grams. For all animals, the test material was administered after an 18-hour fast. The blood samples for glucose determination by the Nelson modification of the Folin-Wu micromethod (3) were withdrawn from the marginal ear vein immediately before, and at five, ten, twenty, thirty, forty, fifty, sixty and ninety minutes as well as at two, three and four hours after the injection. The animals were divided into three main groups:

Group 1. Rabbits untreated with cortisone. These 132 animals were sub-divided as follows: a) Twenty-four groups of 4 animals each received *intravenously* 5, 25 or 50 micrograms of amorphous glucagon, either alone or together with 2, 5, 10, 20, 25, 50 or 100 units of glucagon-free insulin.[1] b) Six groups of 4 animals each received *subcutaneously* 125, 250 or 500 units of either commercial regular insulin (Lilly) or glucagon-free insulin. c) Three groups of 4 animals each received *subcutaneously* 125, 250 or 500 units of protamine zinc insulin (Lilly).

Group 2. Cortisone-pretreated rabbits. These 132 rabbits were pretreated with 50 mg. of cortisone acetate in aqueous suspension (Upjohn)[2] intramuscularly daily for ten days and then divided as follows: a) Twenty-four groups of 4 animals each received *intravenously* 5, 25 or 50 micrograms of amorphous glucagon either alone or together with 2, 5, 10, 20, 25, 50 or 100 units of glucagon-free insulin. b) Seven groups of 4 animals each received *subcutaneously* 50, 125, 250 or 500 units of either commercial regular insulin (Lilly) or of glucagon-free insulin. c) Three groups of 4 animals each received *subcutaneously* 125, 250 or 500 units of protamine zinc insulin (Lilly).

Group 3. Alloxan diabetic rabbits (no cortisone). These 6 animals had been treated with 100 mg. per kilogram of alloxan monohydrate (Eastman) intravenously as 5 per cent solution in distilled water, and had maintained a blood sugar level above 160 mg. per 100 ml. They were utilized on the fifth day after the injection. Each rabbit received a single *intravenous* injection of 500 units of commercial regular insulin (Lilly).

RESULTS

Group 1. Rabbits untreated with cortisone

a. When glucagon alone was administered *intravenously* to untreated rabbits there was in each instance a rise in the level of blood sugar. Although the height of the initial hyperglycemia tended in general to be greater with larger amounts of glucagon, the variability was such that it

[1] The amorphous glucagon was Lot No. 208-105B-197 and the glucagon-free insulin was Lot No. 499667, kindly supplied by the Eli Lilly Co., Indianapolis, Ind., through the courtesy of Dr. Otto K. Behrens.

[2] Cortisone acetate in aqueous suspension was kindly supplied by the Upjohn Company, Kalamazoo, Mich., through the courtesy of Dr. C. J. O'Donovan.

could not be correlated with glucagon dosage. For the three dosage levels, the maximum blood sugar value was achieved at five to thirty minutes and the amount of the increase over the fasting level varied from 13 mg. to 86 mg. per 100 ml., with a combined mean increase of 44 mg. per 100 ml. In most instances a return to the fasting value had occurred by two hours, and in some instances the value tended to dip below the fasting level. When insulin was given together with the various doses of glucagon *intravenously* the blood sugar response varied from an immediate decline to an initial transitory hyperglycemia similar to that observed with glucagon alone. The blood sugar level then decreased rapidly and in all instances had declined below the fasting value within forty minutes. A marked hypoglycemia was then maintained for the duration of the time of the experiment.

b. Commercial regular insulin administered *subcutaneously* in most instances did not cause an initial hyperglycemia, regardless of dosage. In an occasional animal there was observed an initial transient increase of the blood sugar level, up to 28 mg. per 100 ml. over the fasting value. With the glucagon-free insulin, no hyperglycemia was noted in any of the animals.

c. Protamine zinc insulin administered *subcutaneously* to the untreated animals yielded results similar to those observed with regular insulin given subcutaneously.

Group 2. Cortisone-pretreated rabbits

a. In Table 1 may be seen the data for the maximal hyperglycemia achieved with glucagon alone and with combined glucagon and insulin at various dosage levels. The variation between individual values is considerable. However, when the mean of each column is plotted against the logarithm of the glucagon dosage a straight line is obtained. Thus it is apparent that the height of the hyperglycemia achieved was proportional to the increase in the glucagon dosage (Fig. 1). On the other hand, an increase in insulin dosage from 2 to 100 units did not cause a decrease in the degree of maximal hyperglycemia achieved (Table 1). The time of return of the blood sugar level to the fasting value was shorter when insulin was added to glucagon than when glucagon was given alone. Nevertheless, no increase in this effect was observed when the insulin dosage was increased from 2 to 100 units. Similarly a marked secondary reduction in the blood sugar level was observed only when insulin was added to the glucagon. The hypoglycemic level eventually achieved was the same at all insulin dosage levels.

b. A dose of 125 units of commercial regular insulin administered subcutaneously to cortisone-pretreated rabbits caused an initial rise in the blood sugar level from 77 mg. to 166 mg. per 100 ml. (mean, 136 mg.) over

TABLE 1. THE RELATION OF THE DOSE OF GLUCAGON AND OF GLUCAGON-FREE INSULIN TO THE MAXIMAL HYPERGLYCEMIA OBSERVED IN CORTISONE-PRETREATED RABBITS, INDICATING AN INCREASE IN THE LEVEL OF BLOOD SUGAR WITH INCREASING GLUCAGON DOSAGE AND A LACK OF DEPRESSIVE EFFECT WITH INCREASING INSULIN DOSAGES

Glucagon-free insulin (units)	*Glucagon*					
	Dose, 5 μg.		Dose, 25 μg.		Dose, 50 μg.	
	Incr. in blood sugar*		Incr. in blood sugar*		Incr. in blood sugar*	
	Range	Mean	Range	Mean	Range	Mean
0	50 to 90	79	114 to 145	130	165 to 228	208
2	43 to 96	68	59 to 65	62	94 to 203	129
5	46 to 89	70	74 to 141	104	65 to 115	96
10	27 to 152	68	90 to 143	110	57 to 175	131
20	30 to 114	83	106 to 146	128	90 to 143	144
25	63 to 92	78	108 to 118	112	103 to 304	198
50	50 to 92	67	61 to 82	73	122 to 140	131
100	43 to 136	72	90 to 137	115	102 to 210	153

* Increase in concentration of blood sugar (mg. per 100 ml.) above the fasting level.

the fasting values. The peak was somewhat delayed compared to that observed following intravenously injected insulin (Fig. 2). The blood sugar concentration then declined, to reach the fasting level at three and four hours. Increasing the dose of insulin increased somewhat the height of the initial hyperglycemia, and the time of return to normal was further delayed. The eventual hypoglycemic level achieved was the same with all three insulin dosages. A dose of 500 units of glucagon-free insulin administered subcutaneously to cortisone-pretreated animals caused a mild hyperglycemia (Fig. 2). Smaller dosages of glucagon-free insulin caused an

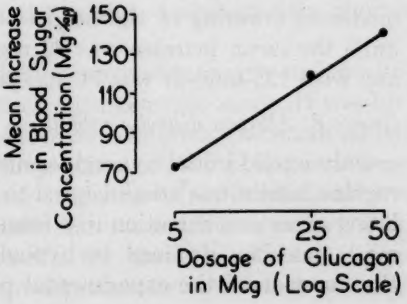

FIG. 1. Relation of the increase in glucagon dosage to the mean increase in blood sugar level in cortisone-pretreated rabbits. The points plotted are the mean maximum rise in blood sugar level observed with dosages of 5, 25 and 50 micrograms of glucagon in combination with dosages of 2 to 100 units of glucagon-free insulin.

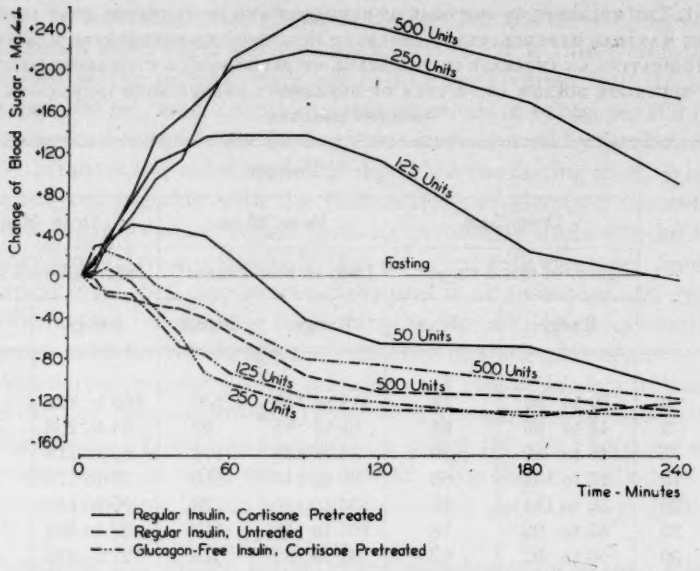

FIG. 2. Effect of the subcutaneous administration of various dosages of commercial regular insulin and of glucagon-free insulin on the mean blood sugar level in cortisone-pretreated rabbits.

immediate decline of the blood sugar concentration without a transient initial hyperglycemia.

c. The subcutaneous administration of 500 units of protamine zinc insulin to cortisone-pretreated rabbits also resulted in an initial hyperglycemia which achieved a maximum increase of 67 mg. to 213 mg. per 100 ml. (mean, 105 mg.) above the fasting value at forty to sixty minutes. The concentration of blood sugar then began to decline and in some instances had returned to the fasting value by two to four hours. In other instances it was still elevated at four hours, at which time the experiment was discontinued. The use of smaller dosages of protamine zinc insulin resulted in moderate lowering of the height of the initial hyperglycemia. With 250 units the mean increase was 82 mg. per 100 ml. (range, 45 to 157 mg.), and with 125 units it was 71 mg. per 100 ml. (range, 40 to 100 mg.).

Group 3. Alloxan diabetic rabbits

Only a mild initial hyperglycemic effect was observed when commercial regular insulin was administered to alloxan diabetic animals (Fig. 3). The blood sugar concentration rose from 5 mg. to 30 mg. per 100 ml. (mean, 12 mg.) and then declined to hypoglycemic levels, where it remained for the duration of the experimental period.

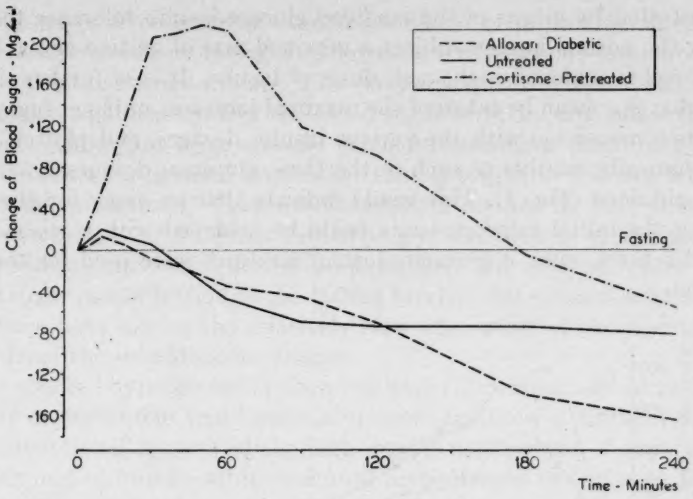

FIG. 3. Effect of the intravenous administration of 500 units of commercial regular insulin on the mean blood sugar level in alloxan diabetic, in cortisone-pretreated, and in normal rabbits.

DISCUSSION

The hypothesis derived from our previous work (1), which suggested that the insulin-antagonistic action ascribed to cortisone is at least in part due to its potentiation of the action of the small amounts of glucagon present in the insulin preparations, appeared to be verified in the present study (Fig. 4). It was demonstrated that the administration of glucagon alone causes an increase in the blood sugar level which is much greater in cortisone-pretreated than in normal animals. Other workers have stated that intravenous glucagon did not cause greater elevation of the blood glucose level in rabbits which had been pretreated with cortisone (10 mg. twice daily) subcutaneously for three days, than it did in normal animals (4). The discrepancy between their findings and ours might be explained by the different mode of administration, the smaller dosage and the shorter period of pretreatment used by them.

When glucagon was added to varying amounts of glucagon-free insulin, the results obtained were similar to those with glucagon alone. It was furthermore observed that in cortisone-pretreated animals the maximal hyperglycemic response was in general proportional to the glucagon dosage. It was of interest that increasing doses of glucagon-free insulin added to the glucagon in a range of 2 to 100 units did not result in a proportional diminution in the hyperglycemic action of the glucagon (Table 1). This is in accord with the results of previous experiments in which the authors

demonstrated by means of the modified glucose-insulin tolerance test (5) that in the normal human subject a maximal rate of decline of the blood sugar level is achieved with small doses of insulin. It is of further significance that if a mean be taken of the maximal increases in blood sugar concentration associated with the various insulin dosages, and plotted semilogarithmically relative to each of the three glucagon dosages, a straight line is obtained (Fig. 1). This would indicate that an assay for glucagon utilizing the initial hyperglycemia could be achieved with cortisone-pretreated rabbits, even if glucagon-insulin mixtures were used for the test material.

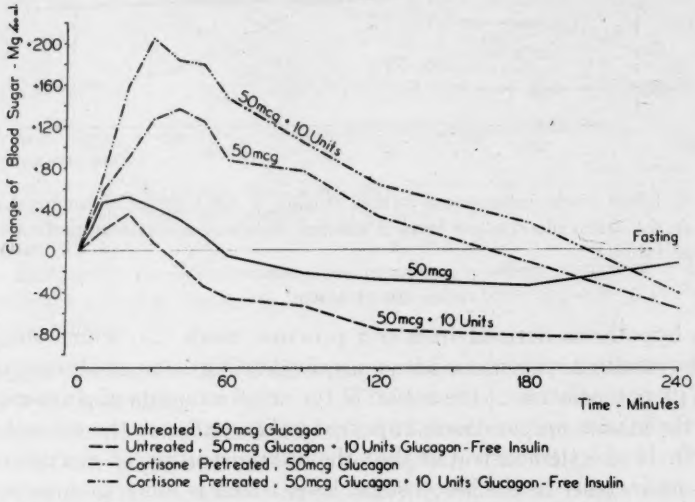

FIG. 4. Effect of the intravenous administration of 50 micrograms of glucagon (either alone or combined with 10 units of glucagon-free insulin) on the mean blood sugar level of normal and of cortisone-pretreated rabbits.

In normal animals, an increase in glucagon dosage had comparatively little effect on either the extent of hyperglycemia, time of return to the fasting value, or the time at which maximal hypoglycemia was achieved. This is in accord with the report that addition of glucagon to insulin does not influence the assay of insulin when performed either by the rabbit or mouse method (2). The results obtained with the alloxan diabetic rabbits indicate that it is not the diabetic state induced by pretreatment with cortisone which accounts for the marked hyperglycemic response. It is interesting that, if anything, the alloxan diabetic rabbit is even less sensitive to the hyperglycemic action of regular insulin than is the normal animal.

Our studies show, furthermore, that the subcutaneous administration

of large doses of commercial regular insulin or of protamine zinc insulin also causes significant initial hyperglycemia in cortisone-pretreated rabbits, but not in normal rabbits. This compares with the report that in normal dogs a significant rise of the blood sugar level did not follow the subcutaneous injection of pancreatic extracts containing denatured insulin (6), and with the general impression that glucagon is inactive when injected subcutaneously. These results confirm further the fact that cortisone potentiates or unmasks the hyperglycemic action of the glucagon contained in commercial insulin preparations. The delayed return of the blood sugar concentration to the fasting level in this series of animals may well have been due to the relatively slow absorption of the injected material from the subcutaneous tissues.

The marked hyperglycemia observed with subcutaneously administered regular or protamine zinc insulin also seems to throw a different light on the conventional concept of the high insulin requirement of experimental animals and of human subjects during corticotropin or cortisone therapy (7–10). Thus, it has been shown that 1000 units of regular insulin or 100 units of protamine zinc insulin were insufficient for complete control of the diabetes induced in rats by administration of 5 mg. of cortisone daily (11). It may be hypothesized that the insulin resistance seen in these circumstances is secondary not only to increased gluconeogenesis but also, at least in part, to the potentiation by cortisone therapy of the hyperglycemic action of the glucagon present in the insulin preparations used, thus preventing the abolition of glycosuria. A similar mechanism may also explain in part the increased resistance to the convulsive action of insulin exhibited by fasted mice treated with carbohydrate-active steroids (12). Moreover, in previous work the authors, utilizing the modified glucose-insulin tolerance test (13), observed a diminution of the rate of decline of the blood sugar concentration in cortisone-pretreated and corticotropin-pretreated dogs as compared with the normal (14). This phenomenon again may have been due to the minute amounts of glucagon contained in the insulin preparations used. The observations presented here suggest, therefore, that the administration of glucagon-free insulin may minimize the increased insulin requirement frequently observed during steroid therapy.

The diabetogenic effect of increased amounts of exogenously administered or of endogenously produced adrenal steroids in animals or in human subjects has been well documented in the literature (15–20). Although there is no proof of a physiologic action of glucagon, it may be that both human and experimental steroid diabetes is due not only to the direct diabetogenic properties of the adrenocortical steroids, but also partially to an additional factor—namely the unmasking of the action of endogenously produced glucagon in the presence of excessive amounts of carbo-

hydrate-active adrenocortical steroids. This idea seems to be negated by the known diabetogenic action of these steroids in the absence of the pancreas. However, further studies to evaluate this tentative hypothesis might be of value.

SUMMARY

In cortisone-pretreated rabbits the intravenous administration of glucagon, either alone or together with various dosages of glucagon-free insulin, caused a marked initial hyperglycemia, the height of which was proportional to the glucagon dosage. On the other hand, a stepwise increase of the amount of insulin in the mixture from 2 to 100 units, did not result in a proportional diminution in the hyperglycemic action of the glucagon.

These findings imply that the cortisone-pretreated rabbit can be utilized for assay of the glucagon content of glucagon-insulin mixtures.

It has been shown, furthermore, that the paradoxical hyperglycemic effect of insulin in cortisone-pretreated rabbits is not due to the diabetic state *per se*, since only a minimal or moderate hyperglycemia was observed in alloxan diabetic animals after large intravenous doses of regular insulin. A marked hyperglycemic effect after cortisone-pretreatment was also obtained with subcutaneously administered commercial regular insulin and with protamine zinc insulin.

It is suggested, therefore that the insulin resistance of human and of experimental subjects induced by excessive amounts of endogenous or exogenous adrenal steroids may be in part the result of the potentiating effect of these steroids on the action of the glucagon contained in the insulin preparations. Thus, it may be advantageous to utilize glucagon-free insulin preparations in the treatment of various conditions in which hyperadrenocorticism is a factor.

REFERENCES

1. Volk, B. W.; Lazarus, S. S., and Lew, H.: The effect of large doses of regular and of "glucagon-free" insulin on the blood sugar of rabbits pretreated with cortisone, ACTH and growth hormone, *Metabolism* **4**: 503–512, 1955.
2. Root, M. A.; Ellis, J., and Staub, A.: Effect of glucagon on insulin hypoglycemia, *Proc. Soc. Exper. Biol. & Med.* **85**: 507–511, 1954.
3. Nelson, N.: Photometric adaption of Somogyi method for determination of glucose, *J. Biol. Chem.* **153**: 375–380, 1944.
4. Helmer, O. M., and Root, M.: The effect of ACTH and cortisone on the hyperglycemic response to glucagon, *Endocrinology* **54**: 338–342, 1954.
5. Volk, B. W., and Lazarus, S. S.: The effect of varying insulin dosages on the rate of decline of the blood sugar in the modified glucose insulin tolerance test, *J. Lab. & Clin. Med.* **41**: 684–689, 1953.
6. Pincus, I. J.: A hyperglycemic factor extracted from the pancreas, *J. Clin. Endocrinol.* **10**: 556–571, 1950.

7. Boland, E. W., and Headley, N. E.: Effects of cortisone acetate on rheumatoid arthritis, *J.A.M.A.* **141:** 301–308, 1949.
8. Sprague, R. G.; Power, M. H.; Mason, H. L., and Cluxton, H. E.: Metabolic effects of synthetic compound E (17-hydroxy-11-dehydrocortisone) in 2 patients with Addison's disease and in 1 with coexisting Addison's disease and diabetes mellitus, (abstract) *J. Clin. Invest.* **28:** 812, 1949.
9. Markovitz, M.: Coexisting diabetes mellitus and Addison's disease; report of two cases, *Metabolism* **3:** 268–273, 1954.
10. Fajans, S. S.; Louis, L. H., and Conn, J. W.: Metabolic effects of ACTH upon pre-existing diabetes mellitus, *J. Clin. Endocrinol.* **11:** 455–464, 1951.
11. Ingle, D. J.; Sheppard, R.; Evans, J. S., and Kuizenga, M. H.: A comparison of adrenal steroid diabetes and pancreatic diabetes in the rat, *Endocrinology* **37:** 341–356, 1945.
12. Gratten, J. F., and Jensen, H.: The effect of the pituitary adrenocorticotrophic hormone and of various adrenal cortical principles on insulin hypoglycemia and liver glycogen, *J. Biol. Chem.* **135:** 511–517, 1940.
13. Lazarus, S. S., and Volk, B. W.: Estimation of insulin sensitivity by the modified glucose insulin tolerance test, *J. Lab. & Clin. Med.* **39:** 404–413, 1952.
14. Volk, B. W.; Lazarus, S. S., and Lew, H.: Effect of various hormones on the rate of decline of the blood sugar in the modified glucose insulin tolerance test, *Metabolism* **4:** 10–18, 1955.
15. Long, C. N. H.; Katzin, B., and Fry, E. G.: The adrenal cortex and carbohydrate metabolism, *Endocrinology* **26:** 309–344, 1940.
16. Ingle, D. J.: The production of glycosuria in the normal rat by means of 17-hydroxy-11-dehydrocorticosterone, *Endocrinology* **29:** 649–652, 1941.
17. Conn, J. W.; Louis, L. H., and Johnston, M. W.: Studies upon mechanisms involved in the induction with adrenocorticotropic hormone of temporary diabetes mellitus in man, *Proc. Am. Diabetes A.* **8:** 213–239, 1948.
18. Bunim, J. J.; Kaltman, A. J., and McEwen, C.: Diabetogenic effect of cortisone and ACTH in a non-diabetic patient with rheumatoid arthritis, *Am. J. Med.* **12:** 125–134, 1952.
19. Sprague, R. G.; Priestley, J. T., and Dockerty, M. B.: Diabetes mellitus without other endocrine manifestations in a case of tumor of the adrenal cortex, *J. Clin. Endocrinol.* **3:** 28–32, 1943.
20. Sprague, R. G.; Hayles, A. B.; Power, M. H.; Mason, H. L., and Bennett, W. A.: "Steroid diabetes" and alkalosis associated with Cushing's syndrome: report of case, isolation of 17-hydroxycorticosterone (compound F) from urine, and metabolic studies, *J. Clin. Endocrinol.* **10:** 289–306, 1950.

RADIOISOTOPE STUDY OF THYROID FUNCTION IN 21 MONGOLOID SUBJECTS, INCLUDING OBSERVATIONS IN 7 PARENTS

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MONGOLISM, a common clinical entity (1) of unknown etiology is believed to result from deceleration of normal growth during the fetal period (2). A possible relationship between abnormalities in development and function of the thyroid gland and the fetal growth process in mongolism is suggested by anatomic evidence of pathologic alterations in the thyroids of mongoloid children (1, 3), and the reported frequency of abnormal thyroid function in their mothers (4).

Although data are available concerning isolated phases of thyroid function in mongolism, a detailed investigation of the various aspects had not, at the time of our studies, been carried out. This report presents the results of an investigation of the thyroid gland in mongolism, utilizing a variety of recently available techniques.

METHOD

Twenty-eight subjects were studied.[1] Twenty-one were mongoloid patients in residence at the Walter E. Fernald State School (Massachusetts Department of Health). Their ages ranged from 5 to 26 years. The remaining 7 were healthy parents (5 mothers and 2 fathers) of mongoloids. Sixty-four euthyroid subjects ranging in age from 10 to 65 years served as controls (5).

In the mongoloid subjects, a dose of 70 microcuries of carrier-free radioactive iodine (I^{131}) was administered orally after an overnight fast. In the parents and in the control subjects, the tracer dose was 100 microcuries.

The thyroidal uptake of the tracer dose was measured by the four-tube G-M method previously described from this laboratory (5), modified by a correction factor for scatter

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[1] Made available through the cooperation of Dr. C. E. Benda, Director of Research, Walter E. Fernald State School.

and absorption which was obtained in each patient by counting a suitable mock-up containing I^{131} in air, and against the patient's neck. Measurements were made twenty-four hours after the tracer dose and on two or more occasions during the subsequent three to five days. The thyroidal turnover of I^{131} reported as the effective half-life (EHL) was calculated according to a previously described method (5, 6). The urinary I^{131} excretion for the first twenty-four hours following the tracer dose was measured by the four-tube method (7).

The plasma butanol-extractable I^{131} (8, 9) (thyroxine-like material) was measured in duplicate at 24-hour intervals for three days following the administration of the tracer dose. The results are reported in per cent as the ratio of radioactivity in the butanol-extractable fraction to the total radioactivity per cubic centimeter of plasma.

Thyroxine metabolism was studied in 2 mongoloids by following the rate of disappearance from the plasma of intravenously infused I^{131}-labeled thyroxine and by measuring the appearance of radioactivity in the thyroid gland after the infusion. The infusion was made with 1.5 micrograms of *l*-thyroxine[2] containing 55 microcuries of I^{131}. Samples of venous blood were obtained daily for ten days. The plasma was separated and 1-ml. aliquots (in triplicate) were counted in a well-type scintillation counter. A standard was prepared from the same shipment of thyroxine-I^{121} and was similarly counted. The plasma radioactivity was plotted daily as a percentage of the administered dose per liter of plasma. After distribution had been achieved, a straight line was drawn which best fitted the data and the half-time of disappearance was calculated. The percentage turnover per day was calculated from the formula: Turnover $= \ln 2/t_{\frac{1}{2}}$. The collection of urine and feces by such patients was not sufficiently reliable to permit more detailed calculations of the rate of synthesis and peripheral degradation of thyroxine (10).

Previous studies from our laboratory (11) had shown that the plasma-thyroid hormone interrelation could be investigated by an *in vitro* system in which the uptake by erythrocytes of I^{131}-*l*-triiodothyronine from whole blood was measured.

Tracer amounts of I^{131}-*l*-triiodothyronine were added to aliquots of whole blood, which were then incubated in a water bath with agitation at 37° C. Following incubation, the erythrocytes were separated, washed, and their radioactive content determined as a percentage of total blood radioactivity, corrected to 100 hematocrit. To assess the respective roles of the plasma and the erythrocytes, "criss-cross" experiments were carried out in which we determined the uptake of I^{121}-*l*-triiodothyronine by mixtures comprising: 1) erythrocytes of mongoloids in compatible plasma of euthyroid subjects, and 2) erythrocytes of euthyroid subjects in compatible plasma of mongoloids.

Labelled thyroxine and triiodothyronine were also added to serum and plasma and the distribution of radioactivity was determined after filter-paper electrophoresis in a Durrum-type[3] cell, using veronal buffer at pH 8.6 and 0.05 u for sixteen hours at 5 milliamperes. After drying in air in an oven for thirty minutes, the strip was scanned for radioactivity by rotating it slowly before a suitable shielded end-window G-M tube (2.4 mg./sq.cm., mica window). Protein was located by staining with bromphenol blue.

RESULTS

Thyroidal 24-hour I^{131} uptake

In the 21 mongoloid subjects, the 24-hour thyroidal uptake (Table 1)

[2] Abbott Laboratories.

[3] Spinco Model R, Spinco Division, Beckman Instruments, Inc.

TABLE 1. DATA ON 21 MONGOLOID PATIENTS

No.	Patient	Age & Sex	Thyroidal 24-hr. I^{131} uptake (%)	Urinary 24-hr. I^{131} excretion (%)	I^{131} half-life (days)	Conversion ratios (%)			PBI, μg./100 ml.	Serum cholesterol (mg./100 ml.)
						24-hr.	48-hr.	72-hr.		
1	R.D.	16 M	17	53	3.6	32	31	69	5.4	175
2	V.B.	16 M	25	49	3.3	38	84	89	5.5	245
3	K.B.	17 M	25	49	8.0	40	67	84	5.4	230
4	G.H.	20 M	30	49	—	38	62	84	7.2	185
5	C.M.	18 M	20	57	2.4	31	50	58	5.1	235
6	K.G.	21 M	25	48	4.2	35	56	68	6.5	195
7	P.H.	25 M	30	45	3.6	51	76	83	6.3	195
8	S.H.	— F	26	22	6.0	43	56	80		—
9	J.B.	18 F	15	28	—	47	62	67	6.2	220
10	M.W.	16 F	12	59	—	35	50	73	4.9	230
11	M.C.	19 F	20	53	6.7	55	84	85	6.5	295
12	E.G.	20 F	31	27	—	—	—	—	6.2	215
13	B.L.	16 F	22	50	7.4	—	—	—	4.7	235
14	M.L.	16 F	25	53	5.7	—	—	—	5.3	230
15	R.T.	27 F	32	41	8.0	—	—	—	8.4	215
16	M.D.	— F	22	42	5.6	13	40	58	—	—
17	M.B.	22 F	26	58	6.3	26	58	67	6.6	205
18	C.D.	— F	30	38	7.5	26	66	63	—	—
19	E.H.	— F	21	52	3.1	16	39	62	—	—
20	J.H.	5 F	26	—	5.8	—	—	—	—	—
21	C.M.	13 F	44	—	6.2	—	—	—	—	—

ranged from 12.0 to 44.0 per cent, with an average of 25.0 per cent (S.E. 1.5; S.D. 6.7).

The mean uptake of the entire group was lower than observed in our previously reported observations of euthyroid subjects (5).

Since the average age of the euthyroid control subjects was higher than that of the mongoloids, another group of 23 euthyroid subjects of an age comparable to that of the mongoloid subjects was studied. The thyroidal uptake values (average, 27.5 per cent; S.E. 1.5; S.D. 7.0) obtained in this group did not differ significantly from those obtained in the larger group of controls ($t = 0.9$). When the mean thyroidal 24-hour I^{131} uptake of the younger group of euthyroid subjests was compared with that of the group of mongoloids, the t value[4] was 1.1—not statistically significant (Table 3).

The thyroidal I^{131} uptake in the 7 parents (5 mothers and 2 fathers) of mongoloid children ranged from 21.0 to 41.5 per cent, with an average of

TABLE 2. THYROIDAL 24-HOUR I^{131} UPTAKE AND TURNOVER IN PARENTS OF MONGOLOIDS

Parent	Age of parent at birth of mongol	Age at time of study	Sex	Thyroidal 24-hr. I^{131} uptake (%)	I^{131} half-life (days)
B.W.	26	27	F	41.5	6.0
E.I.	28–30	31	F	28.4	
E.P.	35	35	F	26.2	7.0
M.H.	46	51	M	26.0	7.5
G.H.	46	51	F	21.0	
H.M.	45	58	F	38.0	4.5
E.M.	42	55	M	35.0	6.2

30.9 per cent (S.E. 2.6; S.D. 6.0). Comparison with data on the group of euthyroid subjects previously reported revealed no significant difference; $t=0.7$ (Tables 2 and 3).

Effective half-life of thyroidal I^{131} (EHL)

The effective half-life of thyroidal I^{131} was measured in 18 mongoloid subjects. The values ranged from 2.4 to 8.0 days, and averaged 5.4 days (S.D. 1.7; S.E. 0.4). In 6 of the 18 patients the EHL was 2.4 to 4.2 days; in the remainder it was 5.6 to 8.0 days. The EHL or thyroidal turnover of I^{131} in mongoloids (Table 3) was significantly faster than in the euthyroid control group ($t=4.0$). No significant difference from the normal ($t=1.9$) was observed in the parents of mongoloids, the EHL ranging from 4.5 to 7.4 days, with an average of 6.2 days (S.E. 0.5; S.D. 1.0).

Urinary 24-hour I^{131} excretion

The 24-hour urinary excretion of I^{131} in the mongoloid subjects ranged from 22 to 59 per cent of the tracer dose. All but 4 values were between 41 and 59 per cent. The average excretion for the entire group was 46 per cent. The urinary excretion of I^{131} in this group of mongoloids did not differ significantly ($t=1.7$) from that of euthyroid controls (Table 3).

Plasma butanol-extractable I^{131} (conversion ratio)

The conversion ratios in the mongoloid subjects (Table 1) ranged from 13 to 51 per cent. Comparable values were obtained in our laboratory in euthyroid subjects (9).

[4] $t = \frac{\text{Difference between the means}}{\text{Standard error of difference between means}}$ and is used as a measure of the statistical significance of the difference between two means. A value of 3.0 or greater is considered significant.

TABLE 3. THYROIDAL 24-HOUR THYROIDAL UPTAKE, TURNOVER, AND I^{121} URINARY EXCRETION IN MONGOLISM

	Mean 24-hr. thyroidal I^{131} uptake			t		Effective half-life			t	Mean 24-hr. urinary I^{131} excretion			t
	%	S.E.	S.D.	A	B	Days	S.E.	S.D.	A	%	S.E.	S.D.	A
A. Euthyroid controls	29.0	0.6	5.1			7.1	0.1	0.8		52		11.9	
B. Mongoloid subjects	25.0	1.5	6.7	2.5		5.4	0.4	1.7	4.0	46		9.9	1.7
C. Euthyroid controls (young adults)	27.5	1.5	7.0	0.9	1.1								
D. Parents of mongoloid subjects	30.9	2.6	6.9	0.7		6.2	0.5	1.0	1.9				

Hormone transport and metabolism

In mongoloid patients, filter-paper electrophoresis was carried out on plasma to which I^{131} thyroxine had been added *in vitro*. In 2 patients, a single peak of radioactivity was found to have migrated with the alpha-2 globulin—an observation comparable to the findings in normal controls. However, the *in vitro* uptake of labelled triiodothyronine by red blood cells was increased. In 9 mongoloids, the uptake ranged from 14.6 to 20.9 per cent and averaged 17.3 per cent. The average value for euthyroid female subjects in our laboratory is 13.0 per cent. In a series of "criss-cross" experiments, the increased uptake was shown to be due to a plasma factor, since erythrocytes from mongoloids had a normal uptake in normal plasma whereas normal erythrocytes had an increased uptake in plasma from mongoloid patients.

Following intravenous infusion of I^{131}-labelled thyroxine in 2 mongoloids, the plasma radioactivity (measured daily for ten days) decreased at the rate of 7.6 per cent and 10 per cent per day, respectively. Similar values have been reported in euthyroid patients (10, 12).

DISCUSSION

The present studies demonstrate the necessity of measurement of multiple parameters in the investigation of thyroid function. Our studies indicate no difference in the 24-hour thyroidal I^{131} uptake, conversion into butanol-extractable material, and urinary I^{131} excretion in mongoloids as compared with euthyroid non-mongoloid subjects of the same age group. Determinations of the serum protein-bound iodine and cholesterol levels (obtained in many of these subjects by Dr. Clemens Benda) (Table 1) were within the range of normal. Isolated studies of thyroid function in mongoloid children found in a review of the literature also revealed no abnormality. One study (13) reported the 24-hour thyroidal I^{131} uptake in a 1-month mongoloid infant to be lower than normal; a second reported a

mongoloid infant (14) with a normal thyroidal 24-hour I^{131} uptake as well as normal values for I^{131} excretion, conversion ratio (TCA precipitation method), and serum protein-bound iodine concentration. Others have found "no significant difference" in serum protein-bound iodine levels between mongoloid children 5 to 15 years old and children of the same age with "undifferentiated mental deficiency" (15). In a recent report of a rare case of thyrotoxicosis in a mongoloid patient, data on 3 other euthyroid mongoloids indicated normal thyroid function (16). Cottino *et al.* (17) found "no endocrine basis for mongolism" after a radioisotope study of 14 patients.

In contrast to these normal findings, the EHL or thyroidal I^{131} turnover was found to be significantly faster in mongoloid subjects than in euthyroid controls. Since the observation of an increased thyroidal I^{131} turnover rate and a normal 24-hour I^{131} uptake might be explained by the assumption that significant turnover and loss of I^{131} by the thyroid had occurred before the 24-hour measurement, we studied 3 mongoloid patients at five and at twenty-four hours after a tracer dose of I^{131}. In these 3 patients, the 5-hour uptake averaged 17 per cent, and the 24-hour uptake averaged 35 per cent. However, since none of these subjects exhibited a rapid turnover, further studies are required.

The apparent discrepancy between rapid thyroidal I^{131} turnover rate and normal serum protein-bound iodine concentration has also not been resolved. If peripheral utilization of thyroid hormone were increased in the same degree as thyroid hormonal release, an equilibrium would be established maintaining the amount of hormone in the blood at a normal level. The normal turnover time of plasma thyroxine observed in 2 mongoloid children does not support the hypothesis of altered peripheral utilization. Since neither of these subjects showed an increased thyroidal turnover of iodide, additional data are needed.

These findings of an increased thyroidal turnover of iodide and a normal plasma protein-bound iodine concentration are consistent with the hypothesis that thyroid function in such individuals resides in only a small portion of the gland working at an increased rate, with consequent normal total iodine uptake, a more rapid turnover, and the effective maintenance of a normal level of serum hormone.[5]

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[5] The diet served to the institutionalized mongoloid patients was obviously not the same as that consumed by our controls. However, the diet was in all respects adequate. Moreover, in New England, with its proximity to the Atlantic, a low-iodine diet is very difficult to achieve. We conceive that the physiologic observation, related to the anatomic abnormalities described by Benda, is an increased turnover rate, so that the total hormonal output by the thyroid remains normal. Further studies would be necessary to prove this point.

Results of anatomic studies (1, 2) of the thyroid gland in mongolism are consistent; the gland is hypoplastic and often below normal weight. Histologic studies reveal additional marked abnormalities. Benda, in a study of 48 mongoloids (1) found only 1 case in which the thyroid gland appeared normal. The main pathologic conditions are described as: 1) resting colloid goiter (most frequent), 2) fetal-type gland with developmental arrest or retardation, 3) replacement fibrosis, and 4) colloid goiter with scattered areas of hyperplasia. It should be emphasized that group 4 represented 21 per cent of the thyroid glands examined.

The other abnormality of thyroid function observed in this study was an increased erythrocytic uptake of triiodothyronine (average 17.3 per cent). Such elevated values have previously been found in thyrotoxicosis. None of these patients had any of the stigmata of hyperthyroidism and, with the exception of thyroidal I^{131} turnover, other parameters of thyroid function were normal. Two additional mentally deficient patients from the same school as the mongoloids (one possibly a cretin) were studied and the erythrocytic uptakes were found to be 11.2 and 9.8 per cent, respectively. Further studies are necessary to clarify the significance of the observation in mongolism, which may indicate an alteration of the plasma protein-thyroid hormone complex or a plasma factor(s) affecting the tissue uptake of thyroid hormone.

Detailed studies of thyroid function in parents of mongoloids are not available. Myers (18) emphasized the significance of thyroid deficiency in the mother. He observed an increase (2:1) in the proportion of mongoloid children born in a geographic area with a high incidence of thyroid disorders as compared to an area with a low incidence. He also reported a markedly higher incidence (9:1 by history) of thyroid disorders in mothers who had given birth to mongoloids as compared to that in mothers of only normal children. By the methods employed, we were unable to detect any abnormality of thyroid function in 7 parents of mongoloid children in our study. However, our studies were made from six months to thirteen years after the birth of the mongoloid offspring and permit no estimation of the maternal thyroid hormonal response to the stress of pregnancy.

SUMMARY

A study of thyroid function in mongolism, utilizing radioisotopic methods of investigation, is reported.

1. In 21 mongoloid subjects, the values for 24-hour thyroidal I^{131} uptake and urinary excretion were in the low-normal range and did not differ significantly from values in a group of euthyroid controls of the same age group.

2. In mongolism, the effective half-life or turnover rate of I^{131} by the

thyroid was significantly faster than in a euthyroid control group. The possible significance of this observation is discussed.

3. The conversion ratio, or the ratio of plasma butanol-extractable I^{131} to total plasma I^{131}, in mongoloid subjects was comparable to that in euthyroid controls.

4. The turnover rate of labelled thyroxine was normal in 2 mongoloid children.

5. Serum cholesterol and serum protein-bound iodine levels were normal.

6. A possible abnormality of the plasma protein-thyroid hormone complex was suggested by the elevated *in vitro* erythrocytic uptake of labelled triiodothyronine in mongolism.

7. No significant abnormality was observed in thyroidal 24-hour I^{131} uptake and turnover or in urinary I^{131} excretion in 7 parents studied six months to thirteen years following the birth of a mongoloid child.

REFERENCES

1. Benda, C. E.: Mongolism and Cretinism, ed. 2. New York, Grune and Stratton, 1949.
2. Benda, C. E.: What is mongolism? *Internat. Rec. Med.* **165**: 75, 1952.
3. Benda, C. E.; Dayton, N. A., and Prouty, R. A.: On the etiology and prevention of mongolism, *Am. J. Psychiat.* **99**: 822, 1943.
4. Benda, C. E.: Prenatal factors in mongolism, *J.A.M.A.* **139**: 979 ,1949.
5. Freedberg, A. S.; Chamovitz, D. L., and Kurland, G. S.: Thyroid function in normal and pathological states as revealed by radioactive iodine studies. I. Thyroid I^{131} uptake and turnover in euthyroid, hyperthyroid and hypothyroid subjects, *Metabolism* **1**: 26, 1952.
6. Freedberg, A. S.; Chamovitz, D. L., and Kurland, G. S.: II. Factors influencing the uptake and turnover of I^{131} by the thyroid gland, *Metabolism* **1**: 36, 1952.
7. Freedberg, A. S.; Ureles, A. L.; VanDilla, M., and McManus, M. J.: A new simple method for accurate measurement of urinary I^{131} after tracer and therapeutic doses, *J. Clin. Endocrinol.* **10**: 437, 1950.
8. Clarke, D. E.; Moe, R. H., and Adams, E. E.: The rate of conversion of administered inorganic radioactive iodine into protein bound iodine of plasma as an aid in the evaluation of thyroid function, *Surgery* **26**: 331, 1949.
9. Hamolsky, M. W., and Freedberg, A. S.: Unpublished data.
10. Ingbar, S. H., and Freinkel, N.: Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis, *J. Clin. Invest.* **34**: 808, 1955.
11. Hamolsky, M. W.: The plasma protein-thyroid hormone complex in thyrotoxicosis vs. euthyroidism in man, *J. Clin. Invest.* **34**: 914, 1955.
12. Sterling, K.; Lashof, J. C., and Man, E. B.: Disappearances from serum of I^{131} labelled L-thyroxine and L-triiodothyronine in euthyroid subjects, *J. Clin. Invest.* **33**: 1031, 1954.
13. Lowry, G. H.; Beierwaltes, W. H.; Lampe, I., and Gomber, H. J.: Radioiodine uptake curve in humans. II. Studies in children, *Pediatrics* **4**: 627, 1949.
14. Kearns, J. E., and Hutson, W. F.: The use of radioactive iodine studies in congenital thyroid aplasia, *Quart. Bull. Northwestern Med. School* **25**: 270, 1951.

15. Simon, A.; Ludwig, C.; Gofman, J. W., and Crook, G. H.: Metabolic studies in mongolism, *Am. J. Psychiat.* **111**: 139, 1954.
16. McGirr, E. M., and Murray, I. P. C.: Thyrotoxicosis in a mongol (Letter to the Editor), *J. Clin. Endocrinol. & Metab.* **16**: 160, 1956.
17. Cottino, F.; Ferraris, G. M., and Coda, G.: Thyroidal function in mongolism (Letter to the Editor), *J. Clin. Endocrinol. & Metab.* **16**: 830, 1956.
18. Myers, C. R.: An application of the control group method to the problem of the etiology of mongolism, *Proc. Am. A. Mental Defic.* **62**: 142, 1938.

EFFECT IN WOMEN OF BILATERAL OOPHORECTOMY ON CIRCULATING THYROID HORMONE*

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CLINICAL and experimental evidence suggests a functional relationship between the gonads and the thyroid. It is recognized that alterations in thyroid function may influence, to some extent at least, ovarian function (1). However, it seems that considerable variation in thyroid function is compatible with normal ovarian function and with reproduction. On the other hand, the influence of gonadectomy (lack of ovarian hormones) or of administered ovarian hormones on thyroid function in animals has been the subject of some debate. Reineke and Soliman (1) recently reviewed the problem and concluded that present evidence indicates that at estrus the metabolic rate is increased, the thyroid appears to be at the point of maximum activity, and the thyroidal uptake of iodine is also at a maximum. Conversely, gonadectomy leads to a decrease in pituitary thyrotropin, and in ovariectomized rats thyroidal iodine uptake is increased by small doses of estrogen administered over a short period. The data support the idea that this action is exerted through the pituitary.

In man, it has been demonstrated by Engstrom and Markardt (2) that large amounts of administered estrogen cause an increase in the concentration of the circulating thyroid hormone, as measured by the level of serum precipitable iodine (SPI). Since normal pregnancy is accompanied by an increased concentration of SPI, it is possible that increased elaboration of estrogen in pregnancy may be an important factor in this rise. On the other hand, no studies are available on the possible effect of sudden oophorectomy in women on the level of the circulating thyroid hormone. Although no definite difference is noted between the SPI level of young women and that of women past the menopause, it is known that a considerable amount of estrogen is elaborated by the menopausal ovary. There-

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fore, the purpose of the present study was to determine the possible acute and chronic effects of bilateral oophorectomy on the level of SPI in women during their reproductive years.

METHODS AND MATERIALS

The concentration of SPI was determined by the digestion-distillation technic as modified by Danowski and associates (3). All determinations were carried out in duplicate on 3-ml. aliquots of serum. In our hands, duplicate determinations check within 0.5 microgram; if not, a third determination is made.

SPI levels were studied in 21 patients before and after bilateral oophorectomy. The ages of these women ranged from 32 to 50 years. In all cases the operation consisted of bilateral salpingo-oophorectomy and total abdominal hysterectomy. Reasons for surgery included uterine fibromyomata, pelvic endometriosis, endometrial carcinoma and benign or malignant ovarian neoplasms. None of the patients received iodine either as medication or for diagnostic purposes before or during the period of study. It is known that the SPI level remains relatively constant in the same individual from time to time; for alterations to be significant the changes must be greater than 1.0 microgram per 100 ml. of serum. It is also known that the stresses of uncomplicated major or minor surgery, in themselves, do not effect the SPI (4).

RESULTS

It may be noted in Figure 1 that all but 1 of the 21 patients had preoperative levels of SPI between 3.5 and 7.0 micrograms per 100 ml. of serum. In

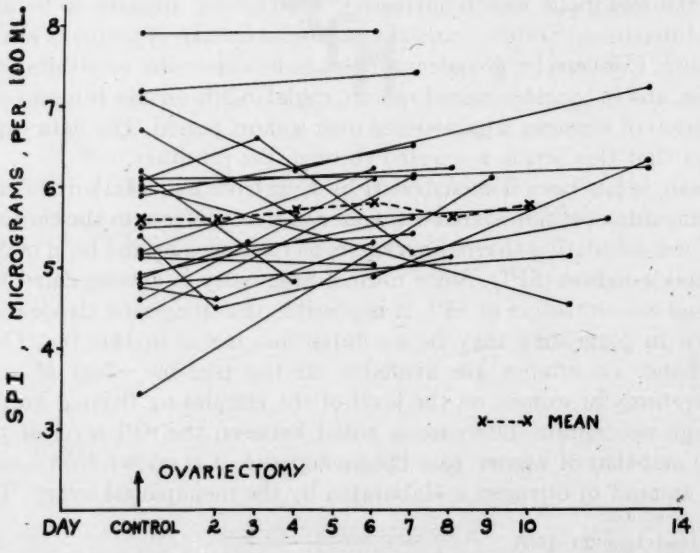

FIG. 1. Course of the serum precipitable iodine level in 21 women during the first fourteen days following bilateral oophorectomy.

our experience, 90 per cent of individuals without thyroid disease have SPI levels in this range. Frequent determinations during the first fourteen postoperative days showed no consistent alteration. In most instances the changes were less than 1 microgram per 100 ml.; in a few cases, increases or decreases were greater than this and were probably greater than the change observed with surgical stress alone (4). This might indicate a transitory flurry of changing thyroidal activity but the alterations were inconsistent in direction.

It was possible to follow 5 of the 21 patients for four to eight months postoperatively. The remainder were lost to the study, either because they

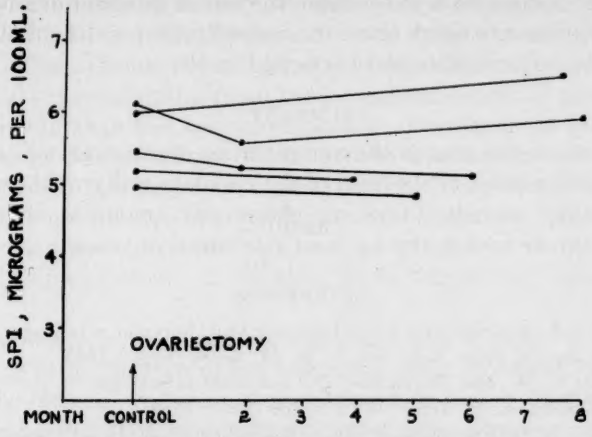

FIG. 2. Course of the serum precipitable iodine level in 5 women for four to eight months after bilateral oophorectomy.

failed to return for SPI determinations or because the severity of the menopausal symptoms necessitated the administration of estrogens. It may be seen in Figure 2 that there was no significant alteration in the SPI levels of these patients.

DISCUSSION

We are not familiar with any other studies on the effect of bilateral oophorectomy on the concentration of SPI in the human subject. In the animal, the effects of gonadectomy on thyroid histology have not been conclusive. Chouke (5) and Starr and Bruner (6) found no change in the histologic picture of the thyroid in female guinea pigs following castration. However, in the rat, Anderson and Kennedy (7) found that, although there was no change in the histologic structure of the thyroid one week after castration, there was definite atrophy in eight weeks. Similar findings were reported by Emge and Laqueur (8).

In man it is thought that hyperthyroidism or hypothyroidism is most apt to occur during the period of changing sexual function. Yet the studies reported here do not indicate any significant variation in circulating thyroid hormone following a surgically induced menopause. Although an increased concentration of circulating thyroid hormone characterizes normal pregnancy and follows the administration of large amounts of estrogen, no opposite change is observed with oophorectomy.

Our studies do not exclude the possibility that gonadectomy may lead to altered responsiveness of tissue to circulating thyroid hormone, or to other changes in thyroid function not measured by the concentration of SPI. If such changes do occur in man, they must be slight or accompanied by other adjustments which allow the concentration of the circulating thyroid hormone to be maintained at normal levels.

SUMMARY

No consistent alteration in the concentration of serum precipitable iodine (SPI), a reliable index of the level of the circulating thyroid hormone, was observed either acutely (days) or chronically (months) after bilateral oophorectomy in women during their reproductive years.

REFERENCES

1. Reineke, E. P., and Soliman, F. A.: Role of thyroid hormone in reproductive physiology in the female, *Iowa State Coll. J. Sc.* **28:** 67–82 (Sept.) 1953.
2. Engstrom, W. W., and Markardt, B.: Influence of estrogen on thyroid function, *J. Clin. Endocrinol. & Metab.* **14:** 215–222 (Feb.) 1954.
3. Danowski, T. S.; Johnston, S. Y., and Greenman, J. H.: Alterations in serum iodine fractions induced by the administration of inorganic iodide in massive dosage, *J. Clin. Endocrinol.* **10:** 519–531 (May) 1950.
4. Engstrom, W. W., and Markardt, B.: The effects of serious illness and surgical stress on the circulating thyroid hormone, *J. Clin. Endocrinol. & Metab.* **15:** 953–963 (Aug.) 1955.
5. Chouke, K. S.: The effect of castration on the proliferative activity and structure of the thyroid gland in guinea pigs, *Endocrinology* **14:** 12–16 (Jan.) 1930.
6. Starr, P., and Bruner, R. C.: Effect of castration on the thyroid in female guinea pigs, *Proc. Soc. Exper. Biol. & Med.* **33:** 465–468 (Dec.) 1935.
7. Andersen, D. H., and Kennedy, H. S.: Effect of gonadectomy on adrenal, thyroid, and pituitary glands, *J. Physiology* **79:** 1–30 (July 28) 1933.
8. Emge, L. A., and Laqueur, G. L.: Thyroid-ovarian relations. I. Influence of ovarian hormones on thyroid hyperplasia, *Endocrinology* **29:** 96–102 (Jan.) 1941.

Teaching Clinic—Review

DIABETES INSIPIDUS*

DIABETES insipidus, a clinical syndrome characterized by the passage of large volumes of dilute urine and the presence of an inordinate thirst, was properly defined one hundred and sixty-two years ago (1). Recently acquired knowledge of the pathogenesis of diabetes insipidus in experimental animals, and the development of a variety of test procedures designed to induce a release of antidiuretic hormone from the pars nervosa of the pituitary, now permit a more precise evaluation of the patient with diabetes insipidus than was heretofore possible. Despite these additions to our knowledge of this syndrome, the differential diagnosis in a patient with polyuria and polydipsia may sometimes be difficult. Improperly conducted test procedures not infrequently contribute to a diagnostic dilemma, and occasionally there are dangerous sequelae from injudicious use of vasopressin (Pitressin) preparations in patients with "suspected" diabetes insipidus.

Some experiences in the diagnosis and treatment of patients with diabetes insipidus form the basis of this communication. However, before discussion of the clinical syndrome, it is pertinent to review briefly some of the experimental data concerning 1) the antidiuretic hormone, and 2) the induction of diabetes insipidus in animals.

THE ANTIDIURETIC HORMONE (VASOPRESSIN)

In 1913 von der Veldin (2) and Farini and Ceccaroni (3) reported that subcutaneous administration of extracts of the posterior pituitary gland corrected the polyuria and polydipsia of patients with diabetes insipidus. These observations were rapidly confirmed, and the fundamental importance of the antidiuretic hormone to water conservation by the kidneys was established (4). Current concepts of the sites of formation, transport and storage of this hormone are derived from the classic studies of the Scharrers, Bargmann, van Dyke, Hild, and others (5–9). These workers demonstrated that neurosecretory material[1] containing vasopressin and

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[1] The neurosecretory material may be stained by several histologic techniques (6, 10) which permit identification in the hypothalamic nuclei and along the axones of the supra-optico-hypophysial tract.

oxytocin first appears in the cell bodies of the supra-optic and paraventricular nuclei, from which it is transported down the supraoptico-hypophysial tract to be stored at the axone terminals of this tract in the pars nervosa (Fig. 1), It is the present consensus that no antidiuretic hormone is formed in the pars nervosa, this portion of the pituitary gland simply serving as a reservoir and site of release for vasopressin and oxytocin.

The mode of action whereby vasopressin effects antidiuresis has not been clarified, but the hormone apparently promotes reabsorption of water by action on the distal renal tubules (4, 11). There is evidence that approximately 85 per cent of the glomerular filtrate (water and solutes) may be reabsorbed in the proximal tubules, and a major portion of the absorption of water from the remaining 15 per cent is believed to be effected by the action of antidiuretic hormone[2] (4). Other mechanisms for reabsorption of water than that influenced by vasopressin are postulated (13, 14). That some active water reabsorption can occur in the absence of antidiuretic hormone is suggested by the observation that animals with experimental diabetes insipidus subjected to dehydration may excrete a hypertonic urine (15). Recently, additional evidence has been presented which clearly demonstrates active water reabsorption in the absence of antidiuretic hormone effect (16).

A variety of stimuli are believed to effect release of antidiuretic hormone from the pars nervosa of the pituitary. Increased serum osmolarity (whether induced by water deprivation or salt administration), pain, fright, unaccustomed exercise, anesthesia, acetylcholine, and nicotine are all capable of provoking a release of vasopressin and consequent antidiuresis in the hydrated animal or man (17–21). Large doses of morphine induce a release of this hormone in the hydrated dog (22), but 20-mg. doses have failed to cause antidiuresis in hydrated humans (23). It is conceivable that simultaneous administration of several suboptimal stimuli, *e.g.*, morphine and anesthesia, may result in summation and cause maximal release of antidiuretic hormone. Apparently alcohol in sufficient amounts *inhibits* release of this hormone from the pars nervosa (24, 25).

The recent synthesis of vasopressin (Fig. 2) by du Vigneaud and associates (26, 27), and the availability of pure preparations now permit investigation of the action of this hormone in various physiologic processes. Van Dyke, Adamsons and Engel (8) have reported that 1 mg. of purified natural vasopressin produced the effect of 600 U.S.P. antidiuretic or pressor

[2] That the action of the antidiuretic hormone does not necessarily result in a concentrated urine of low volume is shown by the studies of osmotic diuresis in normal subjects previously deprived of water. Under these circumstances an increasing rate of urine formation is accompanied by decreasing urine concentration (12).

units as compared with the 1-unit effect of 1 mg. of posterior pituitary powder, U.S.P.[3] Furthermore, in addition to the antidiuretic action, vasopressin induced responses similar to those induced by oxytocin, although of a much lower order of magnitude. This slight oxytocin-like effect is possibly explained by the structural similarity of oxytocin and vasopressin (Fig. 2) (8, 30). Evidence has also been recently presented that vasopressin

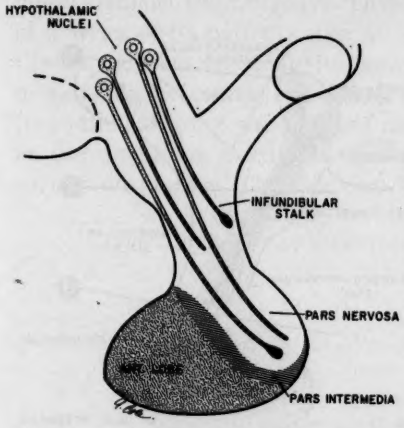

FIG. 1. Diagrammatic representation of neurosecretory material in the cell bodies of the hypothalamic nuclei and in the axones of the supra-optico-hypophysial tract. This material is concentrated at the axone terminals in the pars nervosa (stalk and posterior lobe of the pituitary (5–9)).

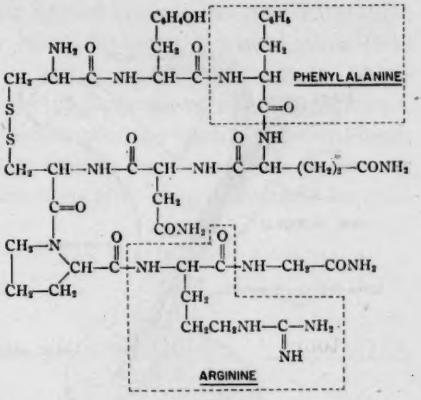

FIG. 2. The structure of vasopressin obtained from the pituitary glands of oxen (arginine vasopressin) (26). Vasopressin from hog pituitary glands (lysine vasopressin) differs in the replacement of arginine by lysine. Oxytocin differs from arginine vasopressin in the replacement of phenylalanine and arginine by isoleucine and leucine respectively (27).

may participate in muscle function (31) and regulation of blood pressure in the human subject (32).

EXPERIMENTALLY INDUCED DIABETES INSIPIDUS

In the experimental animal, diabetes insipidus may be induced by destruction of the hypothalamic nuclei, transection of the infundibular stalk,

[3] The U.S.P. reference standard for posterior pituitary powder assays at 1 unit per 0.5 mg. The antidiuretic hormone may be assayed by several methods. The antidiuresis following subcutaneous injection of test material into hydrated male rats (28) or following intravenous injection into hydrated, unanesthetized, trained dogs (8) are two methods often used. As the ratio of pressor to antidiuretic activity of vasopressin is 1, the hormone may also be assayed by the rise in blood pressure following intravenous injection of test material into anesthetized rats (U.S.P. XV), cats or dogs (29).

or removal of the posterior lobe of the pituitary (15a, 33, 34). Destruction of the hypothalamic nuclei or proximal transection of the infundibular stalk results in a greater polyuria than does removal of the posterior lobe of the pituitary or distal transection of the stalk (Fig. 3) (33, 34). Transection of the supra-optico-hypophysial tract at any level is followed by retrograde degeneration, not only of the divided axones, but also of the respective cell bodies in the supra-optic and paraventricular nuclei (33–35). As

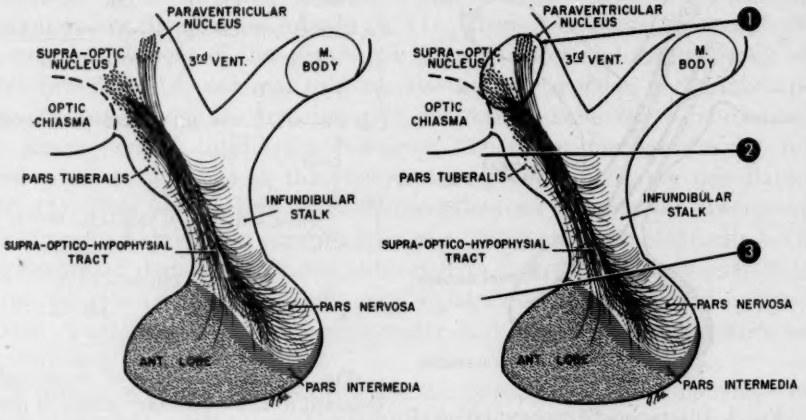

FIG. 3. Diagram of the hypothalamic-neurohypophysial system. Demonstrated on the left is the supra-optico-hypophysial tract which arises from the supra-optic and paraventricular nuclei and terminates in the infundibular stalk and posterior lobe of the pituitary in close relation to vascular channels. Indicated in the figure on the right are the sites of lesions resulting in experimental diabetes insipidus. Destruction of the hypothalamic nuclei (1), or proximal transection of the infundibular stalk (2), is followed by greater polyuria than occurs after removal of the posterior lobe of the pituitary or distal transection of the infundibular stalk (3). (15a; 33–35).

more fibers of the supra-optico-hypophysial tract are divided by proximal than by distal transection of the infundibular stalk (Fig. 3), retrograde degeneration of the supra-optic and paraventricular nuclei is, therefore, greater following the more proximally placed transection. When diabetes insipidus was induced by lesions placed at various levels of the hypothalamic-neurohypophysial system, subsequent cell counts of the supra-optic nuclei revealed that severe diabetes insipidus did not occur until approximately 90 per cent of the cells of these nuclei had been destroyed or damaged (34, 35). Thus, the status of the hypothalamic nuclei, rather than of the posterior lobe of the pituitary, determines the presence or absence of diabetes insipidus. The anatomic studies showing that destruction of the

hypothalamic nuclei is requisite for induction of experimental diabetes insipidus have been given added significance by the demonstration that these nuclei produce the antidiuretic hormone.

DIABETES INSIPIDUS IN MAN

Etiology

Diabetes insipidus may result from any disease in which localized lesions might damage the neurohypophysis or hypothalamus (36–38). In a third of a series of 65 patients seen at the Johns Hopkins Hospital since 1930 (Table 1), diabetes insipidus was due to an intracranial tumor, either primary or metastatic, involving the hypothalamus or neurohypophysis. In another third of the patients no specific etiology could be determined. In the remaining third, the diabetes insipidus was secondary to a wide variety of diseases. That the search for a specific etiology should be con-

TABLE 1. ETIOLOGY OF DIABETES INSIPIDUS IN A SERIES OF 65 PATIENTS

No. of patients	Etiology	Remarks
21	Intracranial tumor	
7	Primary brain tumor	Diabetes insipidus developed postoperatively in 2
5	Chromophobe adenoma	Diabetes insipidus developed postoperatively in 3
5	Craniopharyngioma	Diabetes insipidus developed postoperatively in 4
3	Metastasis from breast carcinoma	
1	Metastasis from osteogenic sarcoma	
22	Unknown	No etiology could be determined
22 7	Hand-Schüller-Christian disease	
6	Hereditary, nephrogenic*	Three of these patients were siblings
3	Post-traumatic	Onset 3 to 21 days following head injury
1	Hereditary†	Not tested for Pitressin sensitivity; many members of family similarly affected
1†	Hereditary	Pitressin-sensitive
1	Leukemia	
1	Rubeola	Onset during febrile phase of rubeola
1	Sarcoidosis	
1	Nonspecific inflammatory reaction	Diagnosis established by operation for suspected brain tumor

* Not true diabetes insipidus, because in this heritable disorder there is no antidiuresis upon administration of Pitressin, thus differing from hereditary† pitressin-sensitive diabetes insipidus.

tinued during the follow-up period was demonstrated by 2 patients in the foregoing series who were considered to have "idiopathic" diabetes insipidus for three and five years, respectively, before the presence of an intracranial tumor in each became recognizable.

Symptoms

The symptoms of diabetes insipidus—polyuria and polydipsia—although remaining relatively constant in any one patient, vary in severity among different patients. In some of the patients listed in Table 1, urine volumes averaged only 4.5 to 5.5 liters daily, but in the majority, urine volumes were in the range of 7 to 11 liters daily. Two factors may explain the difference in urine volumes between otherwise comparable patients: 1) a reduced protein and salt intake decreases the solutes presented to the renal tubules, thus permitting a reduction in urine volume (4, 39, 40); and 2) there is clinical evidence in addition to the previously cited experimental evidence to suggest that diabetes insipidus may result from *partial* as well as total deficiency of antidiuretic hormone. These observations will be discussed subsequently.

In almost all patients in our series the onset of polyuria and polydipsia was abrupt. Most patients could date the onset of symptoms within a one-week or two-week period, and not infrequently the day and hour in which symptoms began were recalled. (A gradually progressive polyuria and polydipsia has been reported to occur occasionally in diabetes insipidus associated with postencephalitic Parkinson's disease (38).)

It is noteworthy that most of the patients with diabetes insipidus expressed a marked preference for iced water and would tolerate much inconvenience to obtain it. When present, this preference for cold water is helpful in the differential diagnosis, for it seldom occurs in patients with primary, or psychogenic, polydipsia. There is no apparent explanation of this desire for iced water in patients with diabetes insipidus, unless it be a reflection of the primacy of polyuria (with dehydration) in this syndrome.[4]

Diabetes insipidus not infrequently follows intracranial surgical procedures directed at the pituitary gland or adjacent areas. The onset of permanent polyuria may be immediate or delayed, but occasionally there is another mode of onset, which has been shown to be characteristic for the diabetes insipidus induced in experimental animals by destruction of the hypothalamic nuclei (15a). Following intracranial surgery there may be, initially, three to four days of polyuria and polydipsia succeeded by several days of normal urine volumes and lack of excessive thirst before

[4] Similarly, a preference for iced water is noted occasionally in patients having polyuria consequent to renal disease.

recurrence of polyuria with the onset of the permanent phase of diabetes insipidus (Fig. 4). This mode of onset has been emphasized recently by Lipsett, Maclean, West, Li and Pearson (41), and it was evident in 4 of the 9 patients with postoperative diabetes insipidus recorded in Table 1. It has been suggested that the "normal interphase" (Fig. 4) may be due to release of preformed antidiuretic hormone from the pars nervosa; then, upon degeneration of the supra-optico-hypophysial tract, permanent diabetes insipidus ensues (34). The previously mentioned concepts of antidiuretic hormone formation and storage support this suggestion (9). In addition, the interphase of normal urine volumes and fluid intake does not

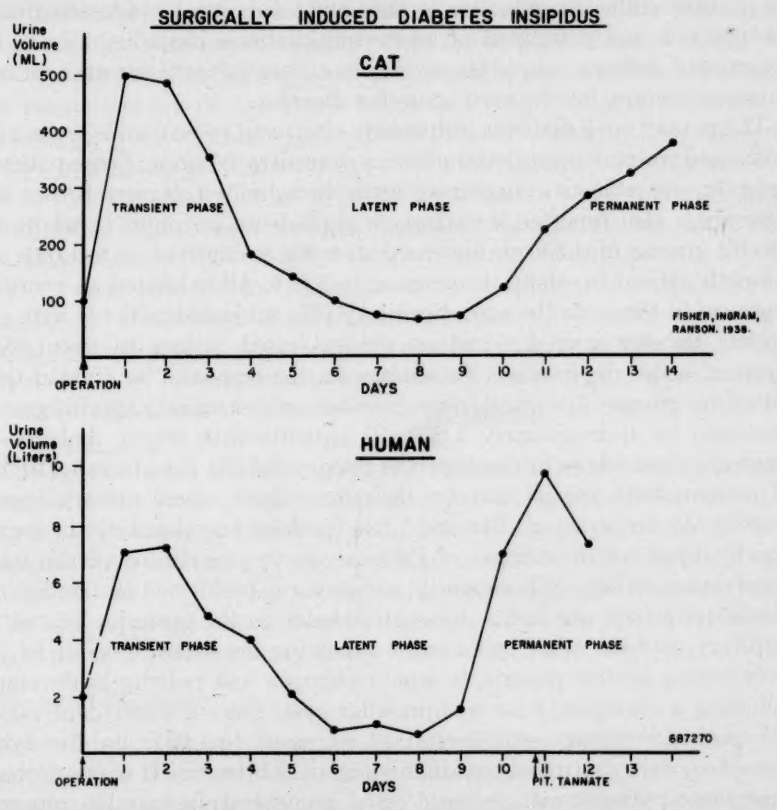

FIG. 4. In the upper diagram are shown the daily urine volumes following operative destruction of the hypothalamic nuclei in the cat (replotted from Fisher, C., Ingram, W R. and Ranson, S. W., ref. 15a). In the lower diagram are shown the urine volumes of a patient following operative removal of a chromophobe adenoma (with inadvertent damage to the hypothalamus or neurohypophysis). The parallelism is striking.

occur in the experimental animal if diabetes insipidus is induced by removal of the posterior pituitary together with the infundibular stalk (34) —a procedure which removes previously formed antidiuretic hormone.

Diagnosis

The diagnosis of diabetes insipidus is usually established on the basis of history, laboratory findings and the patient's inability to form a concentrated urine except after administration of Pitressin. A history of polyuria and polydipsia may not be readily elicited if diabetes insipidus occurs in association with certain other endocrine deficiencies. Anterior pituitary insufficiency (42, 43), hypothyroidism (44) or adrenal insufficiency (45) may induce sufficient reduction in fluid and solute intake to lessen greatly the polyuria and polydipsia of co-existing diabetes insipidus. Hence, the presence of diabetes insipidus may not be suspected until initiation of substitution therapy for the accompanying disorder.

The patient with diabetes insipidus is often said to be unable, even after prolonged water deprivation, to form a concentrated urine. Some patients, however, are able to concentrate urine to a limited degree. Brown and Rynearson (46) reported 3 patients with diabetes insipidus in whom the specific gravity of the urine increased after water deprivation to 1.011, and a fourth patient in whom it increased to 1.016. All exhibited an excellent response to Pitressin therapy. Kourilsky (47) subjected patients with suspected diabetes insipidus and no evident renal disease to twenty-four hours of water deprivation. Depending on the response, he divided them into three groups: 1) normal subjects—those whose urinary specific gravity increased to approximately 1.030; 2) patients with severe diabetes insipidus—those whose urinary specific gravity did not rise above 1.010; and 3) patients with partial diabetes insipidus—those whose urinary specific gravity rose to between 1.010 and 1.024 (with an additional rise in specific gravity upon administration of Pitressin at the conclusion of the water deprivation period). Subsequently, autopsy was performed in 2 patients of this latter group; one had a destructive lesion in the posterior lobe of the pituitary, and the other had a lesion involving the supra-optic nuclei. We have seen a similar patient, in whom polyuria and polydipsia developed following a craniotomy for a suprasellar cyst. During water deprivation, this patient's urinary specific gravity increased to 1.021, and her symptoms, too, were controlled by administration of Pitressin. It seems probable that these patients with reduced renal concentrating function represent examples of *partial deficiency* of the antidiuretic hormone. This evidence of partial deficiency in human subjects is supported by animal studies in which the severity of experimentally induced diabetes insipidus was determined by the location of the lesion.

Although water deprivation may provide useful information, its routine

use as a test procedure for patients with suspected diabetes insipidus has been largely abandoned. In a patient with diabetes insipidus, water deprivation for more than a few hours produces a distressing thirst, and excessive dehydration may lead to alarming symptoms (47, 48). Thus, when subjected to prolonged water deprivation, such patients must be under constant surveillance in order to prevent either surreptitious drinking or excessive dehydration. As the presence of diabetes insipidus can usually be established by other means, this distressing and potentially hazardous procedure seems seldom indicated.

Other procedures devised to test the patient's ability to form a concentrated urine are the intravenous hypertonic saline test of Hickey and Hare (49), and the response to the administration of nicotine (18, 23, 50, 51). Both tests produce an antidiuresis in the hydrated normal subject by causing a release of antidiuretic hormone from the pars nervosa (18, 21, 23). The administration of hypertonic saline fails to induce an antidiuresis in the patient with *severe* diabetes insipidus (49, 52). Unfortunately, this test is often improperly performed and, consequently, subject to misinterpretation (*see* Case report). The intravenous administration of hypertonic saline is contraindicated in most patients with cardiovascular disease. Moreover, the salt load given (0.25 ml. of 2.5 per cent sodium chloride per Kg. of body weight per minute for forty-five minutes) is apparently such a potent stimulus for the release of antidiuretic hormone that patients having a partial deficiency of this hormone may respond much as does the normal subject (41, 50). The response to hypertonic saline may, therefore, be diagnostically misleading in patients with partial antidiuretic hormone deficiency.

The subcutaneous administration of 1 to 3 mg. of nicotine acid tartrate (essentially the same effect is achieved by inhaling the smoke of 1 to 3 cigarettes) also fails to induce an antidiuresis in the patient with diabetes insipidus, but promotes a prompt antidiuresis in the hydrated normal subject (18, 23, 50, 51). This is a simple test procedure and deserves more widespread use, although it is no doubt unwise to administer nicotine parenterally to patients with coronary artery disease (53, 54).

Differential diagnosis

Patients with primary, or psychogenic, polydipsia present the most frequent problem in differential diagnosis. These patients often give a history of gradually increasing polyuria and polydipsia for months or years, in contrast to the abrupt onset of symptoms in most patients with diabetes insipidus. The marked preference for iced water noted in the majority of patients with diabetes insipidus is most unusual in patients with psychogenic polydipsia.

Although these historical aspects are helpful in suggesting the correct

diagnosis, objective criteria are desirable. Most helpful to us in providing such criteria has been a therapeutic test contrasting the effect of a placebo with that of a small dose of Pitressin tannate. An injection of sesame or peanut oil is used as the placebo; in the patient with psychogenic polydipsia there is often a marked reduction in thirst and urine volume, whereas in the patient with diabetes insipidus there is no significant response. The intramuscular administration twenty-four hours later of 0.2 ml. of Pitressin tannate is followed by a prompt reduction in thirst and urine volume in the patient with diabetes insipidus, whereas the patient with psychogenic polydipsia exhibits the same response as to the placebo, or continues to drink large amounts of water despite reduction in urine volume.

Several other syndromes accompanied by varying degrees of polyuria and polydipsia must also be considered in the differential diagnosis. Among these are chronic renal disease (55), potassium deficiency (56), and hereditary nephrogenic diabetes insipidus (57). In the experimental animal, and possibly man (58), excess desoxycorticosterone acetate or cortisone may induce a diabetes insipidus-like state (59–61). In each of these syndromes there is either minimal or no response to administration of Pitressin, and once considered as possibilities, there is usually no problem in diagnosis.

Treatment

The effect of diet on the polyuria of diabetes insipidus is often not considered. Reduction in protein and salt intake has been shown to be an effective means of reducing the urine volume of patients with this disease (39, 62). Such dietary considerations may be important in the occasional patient who takes large amounts of salt, and in the rare patient allergic to Pitressin.

Today Pitressin is most often administered intranasally as powdered pituitary extract or intramuscularly as Pitressin tannate in oil. A dosage of 10 to 20 mg. of powdered extract intranasally administered several times daily provides effective control of symptoms (63, 64); but, as this form of therapy causes nasal irritation in many patients, we have used it only occasionally.

In this clinic the most extensive experience has been with the use of Pitressin tannate in oil. A dosage of 0.2 to 0.3 ml. intramuscularly every one and a half to three days is almost invariably sufficient, and we have not observed any allergic reactions to this preparation. It is insoluble in vegetable oil, so the ampoules should be slightly warmed and must be *shaken vigorously immediately prior to administration.* Each year, 1 or 2 patients are referred because of apparent refractoriness to Pitressin tannate. With a single exception, each of these patients has had the expected response to the drug when it was *shaken immediately prior to injection.* The

single exception was due to a defective preparation, and antidiuresis followed injection of a new "lot number" of Pitressin tannate.

As in any chronic disease, the cost of therapy must be considered. Posterior pituitary powder for intranasal use is available in bulk or, more conveniently, in 45-mg. capsules. Pitressin tannate is available only in 1-ml. ampoules, so it is more economical and convenient to provide the patient with a multiple-dose vial. This can be accomplished by careful transfer of the contents of from five to ten 1-ml. ampoules into a sterile 10-ml. rubber-capped vial. When so supplied, the cost of Pitressin tannate therapy is comparable to that of the capsules of posterior pituitary powder.

Many textbooks recommend 1 ml. of Pitressin tannate (5 units) as the proper single dose. Our experience, as well as that of others (65, 66), indicates that this is a much larger amount than necessary; moreover, in the patient with psychogenic polydipsia, a dose of 1 ml. is not without danger (*see* Case report).

POLYURIA AND POLYDIPSIA—CASE PRESENTATION

Recently seen was a patient whose case history illustrates some of the difficulties encountered in the diagnosis and treatment of patients with polyuria and polydipsia.

M.P. (JHH#168511), a 41-year-old white woman, was seen in the outpatient clinic with the complaints of *progressive polyuria and polydipsia* for twenty years. These symptoms had increased during the preceding year, and urine volumes were estimated by the patient at 10 to 20 quarts daily. She arose four to five times nightly to urinate and, while up, would drink water. *There was no preference for iced water.*

The patient had been recently hospitalized. Noted in the record of that hospitalization were 24-hour urine volumes of 6,000 to 7,200 ml. with an unrestricted fluid intake. Because of an operative procedure, fluids had been restricted during one day to 2500 ml., and the urine volume on this day was *only 950 ml.* Subsequently, an intravenous hypertonic saline test (49) was attempted; as no antidiuresis resulted from the salt administered, *the response was interpreted as indicative of diabetes insipidus.* (In this test the patient received only one fifteenth of the usually recommended amount of sodium chloride; the total amount given over fifteen minutes should have been given over each one-minute period, *i.e.*, 0.25 ml. of 2.5 per cent NaCl per Kg. *per minute* for fifteen minutes.) It was also recorded that the patient had epilepsy with mental retardation and had previously been observed in several episodes of *hysteria.* Relevant laboratory data—concentration of blood sugar 92 mg. per 100 ml., nonprotein nitrogen 25 mg. per 100 ml., serum proteins 6.6 Gm. per 100 ml., and serum chloride 104 mEq. per liter; red blood cell count 5.5 million per cu.mm., specific gravity of urine 1.003, and only an occasional white blood cell in the urinary sediment. Skull roentgenograms and an intravenous pyelogram were unremarkable.

With the foregoing information, and despite the fact that a morning urine brought to the clinic had a *specific gravity of 1.014*, the patient was thought to have diabetes insipidus. Consequently, she was given 1.0 ml.[5] of Pitressin tannate intramuscularly

[5] This dose was recommended in a standard textbook.

and told to return in three days. One and a half days after this injection, the patient was admitted to the hospital with the history that for eighteen hours following the administration of Pitressin tannate she had *continued to drink the usual amounts of water*. Then headache, hiccups and nausea developed. She became stuporous and disoriented, and was admitted to the hospital some hours later in this state.

Physical examination revealed a small, poorly nourished female who was stuporous and combative. The blood pressure was 110/70 mm.Hg, respirations 20 per minute, temperature 99.2° F., and weight 42 Kg. The mucous membranes were moist and, although the patient had taken no fluids during the preceding eighteen hours, she did not seem dehydrated. There was no periorbital or peripheral edema. The neck was flexible, there was no papilledema, and tendon reflexes were slightly hypoactive.

Laboratory data—Urine: specific gravity 1.013, no albumin, and an occasional WBC in the sediment. Blood: concentration of nonprotein nitrogen 25 mg. per 100 ml., sugar 96 mg. per 100 ml., and serum sodium 123 mEq., chloride 87 mEq., and CO_2 combining power 23 mEq. per liter. Cerebrospinal fluid: clear, pressure 110 mm.H_2O, protein concentration 40 mg. per 100 ml., and WBC count 2 per cu.mm.

A diagnosis of water intoxication was made, the explanation being that the patient's excessive water drinking had not been affected by the large dose of Pitressin which had caused a prolonged antidiuresis. For the first twenty-four hours in the hospital, fluid intake was restricted to 600 ml., and during this time the patient excreted 1800 ml. of urine. She then became alert, oriented and cooperative, and the serum sodium and chloride concentrations gradually increased to normal. Subsequently, there was clear-cut antidiuresis following intravenous administration of hypertonic saline (49).

Since discharge from the hospital the patient has cooperated in reducing her daily fluid intake and now is free of excessive thirst and urinary frequency. She has no nocturia, drinks 4 to 6 glasses of liquid daily, and serum electrolyte concentrations are normal.

DISCUSSION OF CASE REPORT

There are several points in this patient's history and hospital record that might well have suggested that she did not have diabetes insipidus. Among these were the slowly progressive nature of the polyuria and polydipsia, no preference for iced water, and one 24-hour urine volume of only 950 ml. with a fluid intake of 2500 ml. That the patient had been observed in several episodes of hysteria was an additional factor to suggest the possibility of psychogenic polydipsia. Although a urinary specific gravity of 1.014 is not necessarily inconsistent with partial deficiency of antidiuretic hormone, it would be unusual to achieve this concentration except after an uncomfortable period of water deprivation. The absence of antidiuresis following intravenous administration of an inadequate amount of hypertonic saline provided misleading information.[6] The final discharge diagnosis of psy-

[6] Other instances of improper procedure in the conduct of this test devised by Hickey and Hare (49) are not infrequent. The continued ingestion of water during infusion of hypertonic saline modifies the antidiuretic response (52) and, in our experience, is the most common precedural error.

chogenic polydipsia was corroborated by the subsequent disappearance of polyuria and polydipsia.

One danger in the use of Pitressin tannate therapy is demonstrated by this case report. Water intoxication was confirmed by both the history and hospital course. Undoubtedly, this would have been less severe had the patient not continued to drink after the onset of symptoms. Water intoxication may also occur in patients with diabetes insipidus (and in normal subjects) who continue to drink, or are forced to drink large amounts of fluid following administration of Pitressin (65–69). In hydrated subjects, a dose of 50 milliunits of Pitressin is sufficient to induce a maximal antidiuretic response, and this antidiuresis may be prolonged by increasing the dosage (70). The increased duration of maximal effect probably accounts for the greater likelihood of water intoxication with 1 ml. of Pitressin tannate (5 units) as compared with smaller amounts.

Additional complications of Pitressin therapy occur occasionally. Pitressin tannate may cause such excessive menstrual bleeding that it must be omitted during menstruation (65). Rarely, the administration of other preparations of posterior pituitary extract is followed by profound hypotension (71, 72) or anaphylaxis (73), but such reactions following injection of Pitressin tannate have not, to our knowledge, been reported.

SUMMARY

In patients with suspected diabetes insipidus, the differential diagnosis frequently taxes the ingenuity of the physician. Helpful in providing a basis for study of such patients is the correlation of current concepts of antidiuretic hormone formation and storage with previously acquired knowledge of the pathogenesis of experimental diabetes insipidus in animals.

Despite an extremely varied etiology, the onset of symptoms in most patients with diabetes insipidus is abrupt. Although, conventionally, diabetes insipidus is considered to be either present or totally absent, there is evidence to suggest that partial deficiency of antidiuretic hormone is clinically recognizable. Some aids in the differential diagnosis of the syndrome of polyuria and polydipsia are reported; among these is the use of a therapeutic test contrasting the effect of a placebo with that of a small amount of Pitressin tannate.

Effective treatment of patients with diabetes insipidus is provided by replacement antidiuretic therapy. When Pitressin tannate in oil is used, excellent control may be obtained with the use of 0.2 to 0.3 ml. intramuscularly every one and a half to three days—a dosage far lower than that recommended in many texts. One patient with psychogenic polydipsia in whom water intoxication developed after the administration of *1 ml.* of

Pitressin tannate illustrates not only the difficulty of correct diagnosis in such patients, but also a serious potential danger in the use of this drug.

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REFERENCES

1. FRANK, J. P.: De Curandis Hominum Morbis Epitome Praelectionibus Academics Dicata, Liber V, De Profluviis, Pars I, Ticini 1794, Ord. I Gen. II, Diabetes, p. 38.
2. VELDIN, R. VON DER: Die Nierenwirkung von Hypophysenextrakten beim Menschen, *Berl. klin. Wchnschr.* **50**: 2083, 1913.
3. a. FARINI, A., and CECCARONI, B.: Pressione arteriosa e diuresi nella terapia ipofisaria, *Clin. med. ital.* (Milano) **52**: 497, 1913.
   b. FARINI, A., and CECCARONI, B.: Influenza degli estratti ipofisari sull' eliminazione dell' acido ippurico, *Gazz. d. osp.* (Milano) **34**: 879, 1913.
4. SMITH, H. W.: The Kidney. Structure and Function in Health and Disease. New York, Oxford Univ. Press, 1951, pages 241–294.
5. a. SCHARRER, E.: Die Lichtempfindlichkeit blinder Elritzen. I. Untersuchungen über das Zwischenhirn der Fische, *Ztschr. vergl. Physiol.* **7**: 1, 1928.
   b. SCHARRER, E., and SCHARRER, B.: Neurosekretion, *in* v. MÖLLENDORFF, W., and BARGMANN, W., editors: Handbuch der Mikroskopischen Anatomie des Menschen. Springer Verlag, Berlin, Göttingen, Heidelberg, 1954, vol. 6, part 5, pp. 953–1066.
   c. LEVEQUE, T. F., and SCHARRER, E.: Pituicytes and the origin of the antidiuretic hormone, *Endocrinology* **52**: 436, 1953.
   d. SCHARRER, E., and SCHARRER, B.: Hormones produced by neurosecretory cells, *in* Recent Progress in Hormone Research, edited by Pincus, G. New York, Academic Press Inc., 1954, vol. 10, pp. 183–240.
6. BARGMANN, W.: Ueber die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse, *Ztschr. Zellforsch. u.mikr. Anat.* **34**: 610, 1949.
7. BODIAN, D.: Nerve endings, neurosecretory substance and lobular organization of the neurohypophysis, *Bull. Johns Hopkins Hosp.* **89**: 354, 1951.
8. VAN DYKE, H. B.; ADAMSONS, K., JR., and ENGEL, S. L.: Aspects of the biochemistry and physiology of the neurohypophyseal hormones, *in* Recent Progress in Hormone Research. New York, Academic Press Inc., 1955, vol. 11, pp. 1–41.
9. HILD, W.: Neurosecretion in the central nervous system, *in* Hypothalamic-Hypophysial Interrelationships, edited by Fields, W. S., Guillemin, R., and Carton, C. A. Springfield, Illinois, Charles C Thomas, Publisher, 1956, pp. 17–30.

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10. Dawson, A. B.: Evidence for the termination of neurosecretory fibers within the pars intermedia of the hypophysis of the frog, *Rana pipiens*, *Anat. Rec.* **115**: 63, 1953.
11. Pickford, M.: Antidiuretic substances, *Pharmacol. Rev.* **4**: 254, 1952.
12. a. Wesson, L. G., Jr., and Anslow, W. P., Jr.: Excretion of sodium and water during osmotic diuresis in the dog, *Am. J. Physiol.* **153**: 465, 1948.
    b. Rapoport, S.; West, C. D., and Brodsky, W. A.: Excretion of solutes and osmotic work during osmotic diuresis of hydropenic man. The ideal and the proximal and distal tubular work; the biological maximum of work, *Am. J. Physiol.* **157**: 363, 1949.
    c. Mudge, G. H.; Foulks, J., and Gilman, A.: Effect of urea diuresis on renal excretion of electrolytes, *Am. J. Physiol.* **158**: 218, 1949.
    d. Seldin, D. W., and Tarail, R.: Effect of hypertonic solutions on metabolism and excretion of electrolytes, *Am. J. Physiol.* **159**: 160, 1949.
    e. Thompson, D. D., and Barrett, M. J.: Urine flow and solute excretion during osmotic diuresis, *Am. J. Physiol.* **176**: 33, 1954.
13. Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics. New York, The MacMillan Co., 1955, page 871.
14. Welt, L. G.: Clinical Disorders of Hydration and Acid-Base Equilibrium. Boston, Mass.; Little, Brown and Co., 1955, page 51.
15. a. Fisher, C.; Ingram, W. R., and Ranson, S. W.: Diabetes Insipidus and the Neuro-Hormonal Control of Water Balance: A Contribution to the Structure and Function of the Hypothalamico-hypophyseal System. Ann Arbor, Michigan, Edwards Bros., Inc., 1938.
    b. Shannon, J. A.: The control of the renal excretion of water. I. The effect of variations in the state of hydration on water excretion in dogs with diabetes insipidus, *J. Exper. Med.* **76**: 371, 1942.
16. Berliner, R. W., and Davidson, D. G.: Production of hypertonic urine in the absence of pituitary antidiuretic hormone, *J. Clin. Invest.* **35**: 690, 1956.
17. Gilman, A., and Goodman, L.: The secretory response of the posterior pituitary to the need for water conservation, *J. Physiol.* **90**: 113, 1937.
18. Burn, J. H.; Truelove, L. H., and Burn, I.: The antidiuretic action of nicotine and of smoking, *Brit. M. J.* **1**: 403, 1945.
19. Pickford, M.: Control of the secretion of antidiuretic hormone from the pars nervosa of the pituitary gland, *Physiol. Rev.* **25**: 573, 1945.
20. Pickford, M.: The action of acetylcholine in the supraoptic nucleus of the chloralosed dog, *Am. J. Physiol.* **106**: 264, 1947.
21. Verney, E. B.: The antidiuretic hormone and the factors which determine its release, *Proc. Roy. Soc., London* **135**: 25, 1947.
22. de Bodo, R. C.: The antidiuretic action of morphine and its mechanism, *J. Pharmacol. & Exper. Therap.* **82**: 74, 1944.
23. Walker, J. M.: The effect of smoking on water diuresis in man, *Quart. J. Med.* **18**: 51, 1949.
24. Rubini, M. E.; Kleeman, C. R., and Lamdin, E.: Studies on alcohol diuresis. I. The effect of ethyl alcohol ingestion on water, electrolyte and acid-base metabolism, *J. Clin. Invest.* **34**: 439, 1955.
25. Kleeman, C. R.; Rubini, M. E.; Lamdin, E., and Epstein, F. H.: Studies on alcohol diuresis. II. The evaluation of ethyl alcohol as an inhibitor of the neuro-hypophysis, *J. Clin. Invest.* **34**: 448, 1955.

26. DU VIGNEAUD, V.; LAWLER, H. V., and POPENOE, E. A.: Enzymatic cleavage of glycinamide from vasopressin and a proposed structure for this pressor-antidiuretic hormone, *J. Am. Chem. Soc.* **75**: 4880, 1953.
27. BARTLETT, M. F.; JOHL, A.; ROESKE, R.; STEDMAN, R. J.; STEWART, F. H. C.; WARD, D. N., and DU VIGNEAUD, V.: Studies on the synthesis of lysine-vasopressin, *J. Am. Chem. Soc.* **78**: 2905, 1956.
28. BURN, J. H.: Estimation of the antidiuretic potency of pituitary (posterior lobe) extract, *Quart. J. Pharm. & Pharmacol.* **4**: 517, 1931.
29. THORP, R. H.: Posterior pituitary lobe hormones, *in* Hormone Assay, edited by Emmens, C. W. New York, Academic Press Inc., 1950, p. 109.
30. DU VIGNEAUD, V.: Trail of sulfur research, *Science* **123**: 967, 1956.
31. INGLE, D. W.: Effect of endocrine glands on normal muscle work, *Am. J. Med.* **19**: 724, 1955.
32. WAGNER, H. N., JR., and BRAUNWALD, E.: The pressor effect of the antidiuretic principle of the posterior pituitary in orthostatic hypotension, *J. Clin. Invest.* **35**: 742, 1956.
33. MAGOUN, H. W.; FISHER, C., and RANSON, S. W.: The neurohypophysis and water exchange in the monkey, *Endocrinology* **25**: 161, 1939.
34. HEINBECKER, P., and WHITE, H. L.: Hypothalamico-hypophysial system and its relation to water balance in the dog, *Am. J. Physiol.* **133**: 582, 1941.
35. MAGOUN, H. W., and RANSON, S. W.: Retrograde degeneration of the supraoptic nuclei after section of the infundibular stalk in the monkey, *Anat. Rec.* **75**: 107, 1939.
36. FINK, E. B.: Diabetes insipidus. A clinical review and analysis of necropsy reports, *Arch. Path.* **6**: 102, 1928.
37. JONES, G. M.: Diabetes insipidus. Clinical observations in forty-two cases, *Arch. Int. Med.* **74**: 81, 1944.
38. BLOTNER, H.: Diabetes insipidus, *in* Oxford Medicine, edited by Christian, H. A. New York, Oxford Univ. Press, 1950, vol. 4, part 1, pp. 179–194.
39. a. MCGAVACK, T. H.; BOYD, L. J., and GELVIN, P.: Experimental modification of water and salt output in patients with diabetes insipidus, *J. Clin. Endocrinol.* **2**: 551, 1942.
    b. BEASER, S. B.: Renal excretory function and diet in diabetes insipidus, *Am. J. M. Sc.* **213**: 441, 1947.
    c. BRODSKY, W. A.; AUSTING, M. E.; MOXLEY, T. L., and GRUBBS, T. A.: Composition of excreted solutes in experimental diabetes insipidus. Mechanism of urinary dilution, *Am. J. Physiol.* **174**: 448, 1953.
    d. DE WARDENER, H. E., and DEL GRECO, F.: The influence of solute excretion rate on the production of a hypotonic urine in man, *Clin. Sc.* **14**: 715, 1955.
40. GAMBLE, J. L., and BUTLER, A. M.: Measurement of the renal water requirement, *Tr. A. Am. Physicians* **58**: 157, 1944.
41. LIPSETT, M. B.; MACLEAN, J. P.; WEST, C. D.; LI, M. C., and PEARSON, O. H.: An analysis of the polyuria induced by hypophysectomy in man, *J. Clin. Endocrinol. & Metab.* **16**: 183, 1956.
42. LEAF, A.; MAMBY, A. R.; RASMUSSEN, H., and MARASCO, J. P.: Some hormonal aspects of water excretion in man, *J. Clin. Invest.* **31**: 914, 1952.
43. HOEL, J.: Cortisone induced recurrence of diabetes insipidus by total destruction of the hypophysis, *Acta endocrinol.* **21**: 15, 1956.

44. BLOTNER, H., and CUTLER, E. C.: Total thyroidectomy in the treatment of diabetes insipidus, *J.A.M.A.* **116**: 2739, 1941.
45. SKILLERN, P. G.; CORCORAN, A. C., and SCHERBEL, A. L.: Renal mechanisms in coincident Addison's disease and diabetes insipidus: effects of vasopressin and hydrocortisone, *J. Clin. Endocrinol. & Metab.* **16**: 171, 1956.
46. BROWN, W. E., and RYNEARSON, E. H.: A procedure for the diagnosis of diabetes insipidus, *Proc. Staff Meet., Mayo Clin.* **19**: 67, 1944.
47. KOURILSKY, R.: Le diabète insipide humain, *Ann. de med.* **48**: 288, 1947.
48. WILLIAMS, R. H.: Textbook of Endocrinology. Philadelphia, W. B. Saunders Co., 1955, p. 682.
49. HICKEY, R. C., and HARE, K.: The renal excretion of chloride and water in diabetes insipidus, *J. Clin. Invest.* **23**: 768, 1944.
50. LEWIS, A. A. G., and CHALMERS, T. M.: A nicotine test for the investigation of diabetes insipidus, *Clin. Sc.* **10**: 137, 1951.
51. CATES, J. E., and GARROD, O.: The effect of nicotine on urinary flow in diabetes insipidus, *Clin. Sc.* **10**: 145, 1951.
52. CARTER, A. C., and ROBBINS, J.: The use of hypertonic saline infusions in the differential diagnosis of diabetes insipidus and psychogenic polydipsia, *J. Clin. Endocrinol.* **7**: 753, 1947.
53. BÜLBRING, E.; BURN, J. H., and WALKER, J. M.: Reduction in coronary flow by pituitary (posterior lobe) extract in relation to the action of nicotine and to smoking, *Quart. J. Med.* **18**: 73, 1949.
54. Ref. 13, p. 622.
55. a. ROUSSAK, N. J., and OLEESKY, S.: Water-losing nephritis; a syndrome simulating diabetes insipidus, *Quart. J. Med.* **23**: 147, 1954.
    b. DARMADY, E. M.; GRIFFITHS, W. J.; MATTINGLY, D.; SPENCER, H.; STRANAK, F., and DE WARDENER, H. E.: Renal tubular failure associated with polyarteritis nodosa, *Lancet* **1**: 378, 1955.
    c. MORGAN, H. G.; FORREST, A. P. M., and LOWE, K. G.: Acquired renal disease simulating diabetes insipidus, *Lancet* **2**: 645, 1955.
56. RELMAN, A. S., and SCHWARTZ, W. B.: The nephropathy of potassium depletion, *New England J. Med.* **255**: 195, 1956.
57. a. FORSSMAN, H.: On hereditary diabetes insipidus, *Acta med. scandinav.* Supplement 159, 1945.
    b. WILLIAMS, R. H., and HENRY, C.: Nephrogenic diabetes insipidus: transmitted by females and appearing during infancy in males, *Ann. Int. Med.* **27**: 84, 1947.
58. MOEHLIG, R. C., and JAFFE, L.: Syndrome simulating diabetes insipidus in dogs induced by desoxycorticosterone acetate, *J. Lab. & Clin. Med.* **27**: 1009, 1942.
59. RAGAN, C.; FERREBEE, J. W.; PHYFE, P.; ATCHLEY, D. W., and LOEB, R. F.: A syndrome of polydipsia and polyuria induced in normal animals by desoxycorticosterone acetate, *Am. J. Physiol.* **131**: 73, 1940.
60. SIREK, O. V., and BEST, C. H.: Intramuscular cortisone administration to dogs, *Proc. Soc. Exper. Biol. & Med.* **80**: 594, 1952.
61. SWINGLE, W. W.; FEDOR, E. J.; BEN, M.; MAXWELL, R.; BAKER, C., and BARLOW, G.: Induction of diabetes insipidus in adrenalectomized dogs with cortisone, *Proc. Soc. Exper. Biol. & Med.* **82**: 571, 1953.
62. CABOT, R. C.: Differential Diagnosis. Philadelphia, W. B. Saunders Co., 1916, vol. 2, p. 533 (Case 234).

63. Smith, F. M.: Diabetes insipidus. Treatment by intranasal insufflation of posterior lobe pituitary powder, *J.A.M.A.* **102**: 660, 1934.
64. Carter, A. C., and Shorr, E.: The intranasal use of posterior pituitary powder in the treatment of diabetes insipidus: a reminder, *J. Clin. Endocrinol.* **7**: 828, 1947.
65. Thorn, G. W., and Stein, K. E.: Pitressin tannate therapy in diabetes insipidus, *J. Clin. Endocrinol.* **1**: 680, 1941.
66. Court, D., and Taylor, S.: Slowly acting pituitary preparations in diabetes insipidus, *Lancet* **1**: 265, 1943.
67. Weir, J. F.; Larson, E. E., and Rowntree, L. G.: Studies in diabetes insipidus, water balance and water intoxication, *Arch. Int. Med.* **29**: 306, 1922.
68. Snell, A. M.: Water intoxication in diabetes insipidus, *Med. Clin. North America* **12**: 1667, 1929.
69. Leaf, A.; Bartter, F. C.; Santos, R. F., and Wrong, O.: Evidence in man that urinary electrolyte loss induced by pitressin is a function of water retention, *J. Clin. Invest.* **32**: 868, 1953.
70. a. Shannon, J. A.: The control of the renal excretion of water. II. The rate of liberation of the posterior pituitary antidiuretic hormone in the dog, *J. Exper. Med.* **76**: 387, 1942.
b. Lauson, H. D.: The problem of estimating the rate of secretion of antidiuretic hormone in man, *Am. J. Med.* **11**: 135, 1951.
71. McMann, W.: Pituitary shock, *Am. J. Obst. & Gynec.* **31**: 1047, 1936.
72. Kantor, A. E., and Klawans, A. H.: Shock from posterior pituitary extract, *Am. J. Obst. & Gynec.* **56**: 366, 1948.
73. McMann, W.: Hypersensitivity to solution of posterior pituitary extract, *J.A.M.A.* **113**: 1488, 1939.

The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

A joint session will be held with The American Goiter Association on Thursday morning, May 30, and a joint session with The American Diabetes Association on Saturday morning, June 1. Both of these joint sessions will be held in the meeting room of The Endocrine Society in the Hotel New Yorker. Admittance of members of the A. G. A. to the Thursday morning session, and of members of the A. D. A. to the Saturday morning session will be by badges of their respective societies.

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

PROGRAM

THURSDAY, MAY 30, 1957

8:30 A.M.—Registration—Grand Ballroom, Hotel New Yorker

JOINT SESSION WITH THE AMERICAN GOITER ASSOCIATION

I. 9:30 A.M.—THYROID

Grand Ballroom, Hotel New Yorker

Chairmen: *E. B. Astwood and J. Howard Means*

1. The Effect of Thyrotropin on the Serum Pattern of Thyroid Hormones. *Jerrold D. Hydovitz* and *Walter L. Arons* (15 minutes).

2. A Quantitative Comparison of the Response to Exogenous Thyrotropin in Euthyroid and Thyrotoxic Human Subjects.
   Monte A. Greer and *Herbert F. Shull* (15 minutes).
3. Distribution of Thyroidal Iodine in Some Nodular Goiters.
   Rosalind Pitt-Rivers, Douglas V. Hubble and *W. H. Hoather* (15 minutes).
4. Metabolites of *l*-Triodothyronine and *l*-Thyroxine.
   Eunice V. Flock, Jesse L. Bollman, John H. Grindley and *Bernard F. McKenzie* (15 minutes).

INTERMISSION

5. Effect of Triiodothyronine and Triiodothyroacetic Acid on Cortisol Production and Catabolism in the Dog.
   James C. Melby, Richard H. Egdahl and *Wesley W. Spink* (introduced by Robert A. Ulstrom*).
6. Interactions of Hydrocortisone and Thyrotropin on the Hormonal Secretion from the Thyroid of Intact Dogs.
   Norman B. Ackerman, J. Martin Miller and *Richmond W. Smith, Jr.**
7. Clinical Studies of an Inhibitor of TSH in Human Urine.
   *Paul Starr** and *Albert Lepp.*
8. Placental Transfer of Thyroxine and Triiodothyronine.
   *E. Knobil** and *J. B. Josimovich.*
9. Studies of Iodinated Compounds in the Blood in Cases of Nontoxic Goiter.
   *J. J. Rupp**, *C. Chavarria, J. Brunet* and *K. E. Paschkis.**

II. 2:00 P.M.—ADRENAL

Grand Ballroom, Hotel New Yorker

Chairmen: *P. H. Forsham and R. I. Dorfman*

10. Further Studies of Aldosterone Induced Hypertension in Rats.
    *A. G. Gornall,** *R. Nakashima, H. M. Grundy, C. J. Koladich* and *M. V. L. Rao.*
11. Changes in the Dog Adrenal Cortex Following Experimentally-Produced Mild or Severe Cardiac Valvular Damage.
    *Helen Wendler Deane** and *A. Clifford Barger.*
12. Relationship of Hypertension to Steroid Formation by Incubated Human Adrenal Tissue.
    *David Y. Cooper** and *Joseph C. Touchstone.**
13. Relationship Between Hydrocortisone Blood Levels, Disappearance Rates and Duration and Degree of Liver Glycogen Deposition Response.
    *E. Myles Glenn, R. O. Stafford** and *B. J. Bowman.*
14. Isolation of a New Steroid from Urine.
    Wojciech Nowaczynski, Thomas Sandor, Erich Koiw and *Jacques Genest.**
15. Some Properties of a Steroid Δ^4-5α-Hydrogenase from Rat Liver Homogenate.
    *Enrico Forchelli** and *Ralph I. Dorfman.**

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* Member of The Endocrine Society.

INTERMISSION

16. ADRENAL CORTICAL CONTROL OF THE 24-HOUR RHYTHM OF ELECTROLYTE EXCRETION IN MAN.
Richard P. Doe, John P. Street, Jack A. Vennes and *Edmund B. Flink.**
17. ADRENAL-THYROID RELATIONSHIPS IN MAN STUDIED BY MEANS OF HYDROCORTISONE-4-C^{14}.
Ralph E. Peterson (introduced by Thomas F. Frawley*).
18. CONCURRENT MEASUREMENT OF PLASMA EPINEPHRINE, NOREPINEPHRINE AND 17-HYDROXYCORTICOSTEROID LEVELS DURING CONDITIONED EMOTIONAL RESPONSE IN MONKEYS.
J. W. Mason, G. F. Mangan, D. G. Conrad and *J. V. Brady* (introduced by M. S. Goldstein*).
19. CORTICOSTEROIDS AND INTERMEDIARY METABOLISM: OBSERVATIONS MADE IN THE INTACT ORGANISM AND IN ISOLATED TISSUES.
*Albert E. Renold,** *E. Rudolf Froesch, Albert I. Winegrad* and *James Ashmore** (invited paper, 20 minutes).
20. FURTHER STUDIES OF PREGNANETRIOL IN THE URINE OF PATIENTS WITH THE ADRENOGENITAL SYNDROME.
*Alfred M. Bongiovanni** and *Walter R. Eberlein.**
21. DEMONSTRATION OF TWO FUNCTIONALLY DISTINCT TYPES OF ADRENAL CORTICAL HYPERPLASIA IN CUSHING'S SYNDROME.
Vincent C. DiRaimondo, S. Hane and *Peter H. Forsham.**

FRIDAY, MAY 31, 1957

III. 9:00 A.M.—REPRODUCTION AND SEX HORMONES

Grand Ballroom, Hotel New Yorker

Chairmen: *C. L. Buxton and L. L. Engel*

22. ON THE REGULATION OF SECRETION OF LUTEOTROPHIN IN THE RAT.
*Irving Rothchild.**
23. STUDIES ON THE MECHANISM OF STIMULATION OF GONADOTROPIN SECRETION USING A NEW RAPID ASSAY.
Albert F. Parlow (introduced by Paul L. Munson*).
24. INDUCTION OF OVULATION IN THE HUMAN: THERAPEUTIC AND DIAGNOSTIC IMPORTANCE.
*Herbert S. Kupperman,** *Jeanne A. Epstein, Meyer H. G. Blatt** and *Abraham Stone.*
25. THE HEPATO-ENTERIC CIRCULATION AND THE METABOLISM OF ESTROGENS IN WOMEN.
*Avery A. Sandberg** and *W. Roy Slaunwhite.*
26. ESTRADIOL-SENSITIVE ISOCITRIC DEHYDROGENASE: STUDIES OF ITS PURIFICATION AND PROPERTIES.
Dwain D. Hagerman and *Claude A. Villee.**
27. NON-STEROID OVARIAN HORMONES. THE EFFECTS OF RELAXIN-CONTAINING PREPARATIONS UPON THE PREGNANT HUMAN UTERUS.
S. Charles Kasdon and *Edward H. Frieden* (introduced by F. Homburger*).

INTERMISSION

28. Isolation and Quantitative Determination of C-21 Steroids with Biological Activity from Human Ovaries.
Josef Zander (introduced by Leo T. Samuels*).
29. The Excretion of Urinary Pregnane 3α-17α-20α-Triol in Pregnancy.
Walter Herrmann (introduced by C. Lee Buxton*).
30. The Effects of Pregnancy and Estrogen on the Metabolism of Corticosteroids.
Ivor H. Mills (introduced by Frederic C. Bartter*).
31. Studies in Women with a New Method for the Fractionation of Urinary 17-Ketosteroids.
Andre Lanthier, Jacques R. Saint-Marc, William J. Reddy** and *George W. Thorn.**
32. Patterns of Hormonal Excretion and Testicular Histology in Ageing.
S. J. Segal, W. O. Nelson** and *R. H. Flocks.*

IV. 1:30 P.M.—ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY

V. 2:30 P.M.—HYPOTHALAMUS, PITUITARY AND PARATHYROID

Grand Ballroom, Hotel New Yorker

Chairmen: *J. A. Russell and C. N. H. Long*

33. Failure of Hypothalamic Tissue to Re-Initiate Thyrotropin Production by Mouse Pituitaries in Tissue Culture.
W. H. Florsheim, M. A. Greer** and *D. T. Imagawa.*
34. Sodium and Potassium Metabolism in Dogs with Ventral Hypothalamic Lesions.
William J. R. Daily and *William P. Ganong.**
35. Concentration of ACTH in Various Parts of the Pituitary Gland.
Guy Rochefort and *Murray Saffran.**
36. The Influence of Glucagon on ACTH Content of Pituitary Glands in Vitro.
A. M. Barrett, Kent Acomb and *George Sayers.**
37. Evidence of an ACTH-Releasing Neurohumor in Peripheral Blood.
A. Brodish and *C. N. H. Long.**

INTERMISSION

38. Aldosterone Stimulating Activity of Extracts of Beef Diencephalon.
Gordon Farrell, Elizabeth W. Rauschkolb, Ronald B. Fleming* and *Frank M. Yatsu*
(invited paper, 20 minutes).
39. Effects of Purified Beef and Monkey Growth Hormone on the Costochondral Junction of Hypophysectomized Monkeys.
E. Knobil, A. Morse, R. C. Wolf,* A. E. Wilhelmi,* D. Phil** and *R. O. Greep.**
40. Effects of Human Growth Hormone in Hypophysectomized Patients.
O. H. Pearson, M. B. Lipsett,* E. Greenberg* and *B. S. Ray.*
41. Mechanisms of Polyuria in Hypophysectomized Man.
J. F. Dingman, A. G. Jessiman, R. H. Despointes, W. G. Hammond, D. D. Matson, K. Emerson, Jr.** and *F. D. Moore.*
42. Hypocalcemic Hypercalcuria During Vitamin D and Dihydrotachysterol Therapy of Hypoparathyroidism.
Jorge Litvak, Marc Moldawer, Anne P. Forbes** and *Philip H. Henneman.**

VI.—ANNUAL DINNER AND PRESENTATION OF THE SOCIETY'S 1957 AWARDS

SATURDAY, JUNE 1, 1957

VII. 9:00 A.M.—DIABETES AND METABOLISM

JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

Grand Ballroom, Hotel New Yorker

Chairmen: *L. W. Kinsell and F. D. W. Lukens*

43. Effect of Growth Hormone on Glucose Production, Utilization and Insulin Response in Hypophysectomized Dogs.
N. Altszuler, R. Steele, J. S. Wall* and *R. C. de Bodo.**
44. Glucose Penetration into Liver.
George F. Cahill, Jr., A. Scott Earle and *James Ashmore.**
45. Glucagon and Renal Function.
H. Elrick, E. Huffman, C. Hlad, N. Whipple and *A. Staub* (introduced by Gilbert S. Gordan*).
46. The Effect of Insulin on Mucopolysaccharide Turnover in Rat Skin.
*Sara Schiller** and *A. Dorfman.*
47. Functional and Morphologic Studies on the Effect of Orinase on the Pancreas.
*Sydney S. Lazarus** and *Bruno W. Volk** (invited paper, 20 minutes).

INTERMISSION

48. Pentose and Glucose Studies Including Carbohydrate Tolerance Using Intravenous Arylsulfonylureas Substituted for Insulin.
Thomas F. Frawley, Stanton Segal, Mary M. Camus* and *Joseph B. Foley.*
49. Effects on Blood Sugar of a New Potent Hypoglycemic Compound.
Robert L. Nielsen, Heidi E. Swanson,* D. C. Tanner** and *Robert H. Williams.**
50. Glucose Utilization by the Leucocytes of Patients with Diabetes Mellitus.
*Mary E. Dumm.**
51. The Adrenal Medullary Response to Insulin-Induced Hypoglycemia
Alan Goldfien, Robert Moore,* Sheref Zileli,* Lenore Boling** and *George W. Thorn.**
52. Effects of Steroids of Non-Corticoid Configuration upon Lipid and Carbohydrate Metabolism.
George D. Michaels and *Laurance W. Kinsell.**
53. Calcium-45 Dynamics in Human Metabolic Bone Disease.
Robert P. Heaney and *G. Donald Whedon.**

VIII. 2:00 P.M.—CLINICAL SESSION

Grand Ballroom, Hotel New Yorker

Chairman: *E. C. Reifenstein, Jr.*

I. *Symposium on Hormone Therapy and Pregnancy*

A. Therapy to Aid Conception.
*E. T. Tyler.**

B. THERAPY TO MAINTAIN PREGNANCY.
*W. M. Allen.**
C. THERAPY TO CONTROL POSTPARTUM BREAST MANIFESTATIONS.
*J. J. Gold.**
D. DISCUSSION.

INTERMISSION

II. *Symposium on Irradiation Therapy for Endocrinopathies*

A. THERAPY FOR ADRENAL DISORDERS.
*J. W. Jailer.**
B. THERAPY FOR GONADAL DISORDERS.
*A. E. Rakoff.**
C. THERAPY FOR THYROID DISORDERS.
*M. Sonenberg.**
D. VIEWPOINT OF THE RADIOLOGIST.
L. H. Garland (by invitation).
E. DISCUSSION.

TO BE READ BY TITLE
(Papers #54–#71)

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman;

1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such ap-

plications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957.*

NINTH INTERNATIONAL CONGRESS ON RHEUMATIC DISEASES

The Ninth International Congress on Rheumatic Diseases will be held in the Royal York Hotel, Toronto, Ontario, Canada, *June 23–28, 1957,* under the auspices of the Canadian Rheumatism Association. All correspondence should be addressed to Post Office Box 237, Terminal "A," Toronto, Ontario, Canada.

COURSES IN PEDIATRICS

The following short refresher courses will be given at The Children's Hospital of Philadelphia in *May and June 1957:*

1. *Pediatric advances for pediatricians and general practitioners.* May 27 through May 31, 1957. Conducted by the Staff of the Children's Hospital of Philadelphia, in collaboration with the Department of Pediatrics of the University of Pennsylvania and the Staff of the Camden Municipal Hospital. Tuition, $110.00.
2. *Practical pediatric hematology.* June 3, 4 and 5. Conducted by Irving J. Wolman,

M.D. and other members of the Hematology Department of the Children's Hospital, under the auspices of the Graduate School of Medicine, University of Pennsylvania. Tuition, $75.00.

3. *Blood group incompatibilities and erythroblastosis fetalis.* June 6 and 7. Conducted by Neva Abelson, M.D. and Thomas R. Boggs, Jr., M.D. of the Philadelphia Serum Exchange of the Children's Hospital of Philadelphia, under the auspices of the Graduate School of Medicine, University of Pennsylvania. Tuition, $50.00.

Inquiries should be addressed to Irving J. Wolman, M.D., Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia 46, Pa.

FOURTH PAN-AMERICAN CONGRESS ON ENDOCRINOLOGY

The Fourth Pan-American Congress on Endocrinology will be held in Buenos Aires, November 3 to 9, 1957, presided over by Prof. Bernardo A. Houssay, and organized by the Argentine Society of Endocrinology and Metabolism.

Membership fees: Active members: $15.00 U. S.
Adherent members: $10.00 U. S.
Associate members: $10.00 U. S.

Communications: Each active member of the Congress will be allowed to submit more than one paper, but will be entitled to present personally a single communication. Authors should send *two* copies of the abstract of the communication not exceeding 260 words, typewritten, double spaced on letter-size paper. No tables or illustrations may be included. At least one of the authors must be registered as an active member of the Congress.

The deadline for the receipt of communications is June 15, 1957.

For further information, booklet, circular, etc. write to: Fourth Pan-American Congress on Endocrinology, Asociacion Medica Argentina, Santa Fe 1171, Buenos Aires, Argentina.

INTERNATIONAL MEDICAL-SURGICAL MEETINGS

From June 1 to 9, 1957, the palace of "Torino-Esposizioni" will again open for physicians, surgeons and specialists in all fields of the medical sciences who will meet here for the Third International Medical-Surgical Meetings—Conference on Scientific Films.

Of world-wide importance will be the International Congress on Nuclear Medicine, the International Congress on Photobiology and the International Congress on the Problems of Goitre. Of the seven national congresses, those on Chemiotherapy, on Gastroenterology and the Spring Session of the National Congress of the Italian Society of Surgery are

worthy of particular note; the International Symposia include such exceptional events as the Symposium of the European Society of Cardiovascular Surgery, the associated Meetings on Cardiosurgery and the Symposium on Artificial Heart-Lung Machines.

Address correspondence to: Segreteria Generale, Minerva Medica, Corso Bramante 83–85, Turin, Italy.

SYMPOSIUM ON RECENT DEVELOPMENTS IN RESEARCH METHODS AND INSTRUMENTATION

Sponsors—The Washington Sections of the American Chemical Society, Instrument Society of America, Society of American Bacteriologists, American Association of Clinical Chemists, Society for Experimental Biology and Medicine, and Professional Group on Medical Electronics of the Institute of Radio Engineers. Held in conjunction with the *Seventh Annual Research Equipment Exhibit, May 13–15, 1957*, at the Clinical Center Auditorium, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland.

STATEMENT BY THE UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION

The Scientific Committee on the Effects of Atomic Radiation established by the United Nations General Assembly accepts the view that the irradiation of human beings, and especially of their germinal tissue, has certain undesirable effects.

Information received so far indicates that, in certain countries (Sweden, United Kingdom, United States of America), by far the most important artificial source of such irradiation is the use of radiological methods of diagnosis and that this may be equal in importance to radiation from all natural sources. It is possible that such radiation may be having a significant genetic effect on the population as a whole.

The Committee is fully aware of the importance and value of the medical use of radiations but wishes to draw the attention of the medical profession to these facts and to the need for a more accurate estimate of the amount of exposure from this source. The help of the medical profession would be most valuable to make it possible to obtain fuller information on this subject.

The Committee would be particularly grateful for information through appropriate governmental channels on ways in which the medical irradiation of the population can be reduced without diminishing the true value of radiology in diagnosis or treatment.

BOOK REGISTER

Low-Fat Cookery. By EVELYN S. STEAD and GLORIA K. WARREN, with an introduction by EUGENE A. STEAD, JR., M.D. and JAMES V. WARREN, M.D. As a measure to help obese persons, heart patients, or diabetics, this book offers a positive way to weight control; 184 pages; 1956. McGraw-Hill Book Company, Inc., New York, N. Y. Price $3.95.

Lymphatics, Lymph and Lymphoid Tissue. By JOSEPH MENDEL YOFFEY, D.Sc., M.D., F.R.C.S., Professor of Anatomy, University of Bristol and FREDERICK COLIN COURTICE, M.A., D.PHIL, D.Sc., F.R.A.C.S., Director, Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney. This book constitutes a thorough revision of C. K. DRINKER and J. M. YOFFEYS' book by the same name, long out of print. It deals with the essential problems of the interior of body cells. To do this involves an up-to-date account of the details of capillary structure, the factors governing capillary permeability, the extra-vascular circulation of protein, and many related topics. Special consideration is given to the lymphatic drainage of various regions and tissues in relation to their function. Lymphoid tissues are dealt with chiefly from the point of view of their function in health. This is the only book of its kind; 510 pages; 99 figures; 1956. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Massachusetts. Price $10.00.

Medical Sciences. Volume 1 of Progress in Nuclear Energy, Series VII. Edited by J. C. BUGHER, The Rockefeller Foundation, New York, J. COURSAGET, C. E. A., Saclay, and J. F. LOUTIT, Medical Research Council, A.E.R.E., Harwell. The various chapters deal with radioactive isotopes in medical diagnosis, the the diagnosis and investigation of disease with radioactive isotopes, nuclear radiations for radiotherapy with external sources, radioactive materials for internal therapy, principles and standards of radiation safety, health and safety activities in reactor and chemical processing plants, and dosimetry of ionizing radiation; 165 pages; 1956. McGraw-Hill Book Co., Inc., New York, and Pergamon Press, London. Price $6.00.

Modern Office Gynecology. By GEORGE BLINICK, M.D., F.A.C.S., Attending in Obstetrics and Gynecology, Beth Israel Hospital, and Assistant Clinical Professor of Obstetrics and Gynecology, New York University College of Medicine, New York, and SHERWIN A. KAUFMAN, M.D., F.A.C.S., Associate Attending in Obstetrics and Gynecology, Beth Israel Hospital, New York. This book is intended for everyday use by both the general practitioner and gynecologist for on-the-spot diagnosis and therapy in office practice. The first section is a discussion of common gynecologic symptoms, with tables for differential diagnosis. The second section consists of a gallery of drawings. The third section consists of a concise and up-to-date review of the more specialized aspects of office gynecology, in the form of a bibliography with abstracts; 218 pages; 47 illustrations; 1957. Lea & Febiger, Philadelphia.

Physiology and Pathology of the Kidney. The British Medical Bulletin, Volume 13, January, 1957. This is a symposium, written by clinicians, which is a sample of work

in progress at the present time. The advances in medicine over the last twenty-five to thirty years largely arise from the application of physiology to clinical medicine. Nature poses certain problems such as renal edema, aminoaciduria and chronic renal failure which are difficult, if not impossible, to reproduce in the experimental animal, and which must therefore be studied in man. Under the chairmanship of Professor Robert Platt of the University of Manchester, a group of distinguished British clinical investigators, pathologists, radiologists, physiologists, biochemists and medical physicists interpret the ways of the kidney in health and disease. Published by the Medical Department, The British Council, 65 Davies Street, London, W.1, England. Price $3.25.

Problems of Aging. Edited by ROBERT L. CRAIG, M.D. A Symposium presented at the Twenty-Eighth Annual Graduate Fortnight of The New York Academy of Medicine, October 10 to 21, 1955. All of the papers have been published serially during 1956 in The Bulletin of the New York Academy of Medicine, and represent the work of prominent investigators in the field of aging. The collection is meant to serve as a reference, not only for the practicing physician, but for those in other fields who may wish to review in concise form the many problems in gerontology; 240 pages, illustrated; 1956. Distributed by George Eliot, Medical and Scientific Books, New York 21, N. Y. Price $3.50.

The Hazards to Men in Ships Lost at Sea, 1940–44. Medical Research Council Special Report Series, No. 291, by R. A. MCCANCE, C. C. UNGLEY, J. W. L. CROSFILL and E. M. WIDDOWSON; paper; 44 pages; 17 tables; 8 figures; 1956. Her Majesty's Stationery Office, London, England. Price 5s. 0d. net.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Administrative Medicine Fourth Conference, Oct. 31–Nov. 2, 1955, Princeton N.J. Edited by GEORGE S. STEVENSON, M.D., National and International Consultant, National Association for Mental Health, New York, N. Y. Contents—Measuring needs for medical and related services, health insurance plan of Greater New York, assessment of quality of services in a group practice prepayment plan, assessing effectiveness of programs in operation, study of the quality of medical care, assessment of quality of services in the Boston Metropolitan area, and British experience in appraising adequacy of medical care; 251 pages; 1956. Price $4.25.

Glaucoma. First Conference, Dec. 5–7, 1955, Princeton, N. J. Edited by FRANK W. NEWELL, M.D., Department of Surgery (Ophthalmology), The University of Chicago, Chicago, Illinois. Contents—A review of angle-closure glaucoma, central control of intraocular pressure, and physiologic and pharmacologic factors influencing the resistance to aqueous outflow: 251 pages; illustrated; 1956. Price $4.50.